Androgens and the Female Brain:
The Relationship between Testosterone Levels,
Depression, Anxiety, Cognitive Function and Emotion
Processing in Females with Polycystic Ovarian Syndrome

Mayouri Sukhapure

A thesis submitted for the degree of

Doctor of Philosophy

at the University of Otago, Christchurch,

New Zealand

October 2018
Abstract

Background
Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder in reproductive-aged females, with the main hormonal abnormality being androgen (testosterone) excess. Although it is well-established that there is a higher prevalence of depression in PCOS, little research has examined the direct relationship between androgen levels, depression and associated cognitive impairment. The current study conducted a simultaneous investigation of the relationship between androgen levels, mood, cognitive function and emotion processing in females with PCOS, before and after 12 weeks of anti-androgen treatment. Elucidating the relationship between androgens and mood in females could have significant benefit in determining optimal treatment for the subset of females whose symptoms of depression may be related to an underlying androgen excess.

Objectives
- To describe differences between the PCOS ($n = 53$) and non-PCOS control ($n = 54$) groups on measures of mood, anxiety, cognitive function and emotion processing,
- To determine whether correlations exist between androgen levels and symptoms of depression and anxiety, cognitive function, and emotion processing in the entire sample with varying androgen levels,
- To determine whether symptoms of depression relate to aspects of cognitive function and emotion processing,
- To determine whether anti-androgen treatment is associated with changes in mood, anxiety, cognitive function and emotion processing in the PCOS group.
Methods
Fifty-three females with PCOS and 54 age-matched females without PCOS completed an assessment of androgen levels from blood samples, depression-rating scales, and cognitive and emotion processing measures at two-time points; baseline and 12 weeks. The PCOS group commenced anti-androgen treatment following baseline assessment.

Results
- Females with PCOS had more symptoms of depression but not anxiety, and showed worse performance on the cognitive domains of psychomotor speed and emotion processing compared with the control group.
- Higher testosterone levels were significantly associated with worse mood and worse cognitive function in sub-tests within the domains of verbal and visuospatial learning and memory, psychomotor speed and emotion processing across the entire sample.
- Significant correlations were found between worse mood and anxiety and poorer cognitive performance on aspects of verbal and visuospatial learning and memory, and attention and executive function measures.
- Anti-androgen treatment was associated with significant improvements in psychomotor speed and aspects of emotion processing in the PCOS group.
- Following anti-androgen treatment, a significant improvement was observed in symptoms of depression and anxiety and on performance on verbal and visuospatial learning and memory and emotion processing measures within the PCOS group.
- Improvement in symptoms of mood was associated with improved performance on measures of psychomotor speed, attention and executive function and improved recognition accuracy of fearful and disgusted faces within the PCOS group.
Conclusions

Females with PCOS showed greater symptoms of depression and worse cognitive function compared with non-PCOS control participants. Additionally, higher testosterone levels were associated with greater symptoms of depression and worse cognitive performance. Anti-androgen treatment appeared to have a significantly beneficial effect on mood and aspects of cognitive function in the PCOS group.

Results from the current study thus provide preliminary evidence that testosterone excess in females with PCOS is associated with greater depressive symptoms and worse cognitive function, which may be benefited by anti-androgen treatment.
Preface

The research presented in this PhD thesis was conducted between December 2013 and October 2018 while I was enrolled as a PhD student at the Department of Psychological Medicine, University of Otago, Christchurch, New Zealand. Professor Richard Porter was my primary supervisor, from the same department. Dr Katie Douglas, also from the same department, co-supervised the project, along with Dr Anna Fenton, Gynaecological-Endocrinologist at the Canterbury District Health Board. My research was funded by a University of Otago Doctoral Scholarship.

Data from the current study were collected between June 2014 and December 2017. Patients with PCOS were recruited from Christchurch Women’s Hospital and a private clinic, and control participants were recruited from the general population in Christchurch. All assessments were conducted at the Department of Psychological Medicine, Christchurch, New Zealand. Ethical approval for the study was obtained prior to the study commencing from the University of Otago Human Ethics Committee (Health).

The following people contributed to the study:

- Professor Chris Frampton (biostatistician) recommended appropriate statistical procedures to analyse data from the current study.
- Bridget Kimber (research nurse) took blood samples from control participants to assess testosterone levels.

I co-ordinated all aspects of the study, which involved:

- Applying for ethical approval
- advertising, screening, recruiting and obtaining informed consent from patients with PCOS and control participants from the general population in Christchurch,
• conducting mood and cognitive assessments for patients with PCOS and control participants, in total, 214 cognitive assessments,
• posting a letter to participants three weeks before the follow-up assessment (at the twelve-week mark) to ensure participation continued,
• organising follow-up assessments,
• arranging for blood samples to be collected for the control group,
• administering appropriate screening questionnaires, structured interviews, and depression and anxiety ratings scales for the PCOS and the control groups, and
• conducting statistical analyses on the data.

Aspects of this research have been presented at the scientific meeting of the Health Research Society of Canterbury (and published in the proceedings), at the Department of Psychological Medicine Research Meetings, University of Otago Open Day presentations, PhD competitions including the three-minute thesis and the AMP Ignite. Additionally, I have been selected to present the main findings from my PhD in the form of an oral presentation at the Society for Mental Health Research (SMHR) in November 2018 in Noosa, Queensland, Australia. Following submission of this PhD, I intend to apply for a University of Otago Publishing Grant to help prepare and publish findings in scientific peer-reviewed journals.
Acknowledgements

This PhD thesis has been my ‘sadhana’- a term used in Yoga, which means a practice of meditative discipline in pursuit of a goal which not only benefits the practitioner but also brings about a wholesome change in the community. Pursuing this doctoral degree has been an exciting, rewarding and challenging journey throughout, for which I wish to thank many.

I have been very fortunate in having received excellent supervision from academics and clinicians of a high caliber, namely Professor Richard Porter, Dr. Katie Douglas and Dr. Anna Fenton. I was lucky to receive their knowledgeable and inspiring guidance while enjoying creative freedom during all stages of the PhD. I would like to express my gratitude to my primary supervisor, Richard Porter, for his immense knowledge, encouragement, and sense of humour when reviewing literature and writing got intense, and for considering me to be worthy of taking on the role of Princess Fiona from Shrek for a PhD competition! I am deeply appreciative of Katie Douglas for her capable, warm and positive supervision. Her supportive guidance prepared me to conduct cognitive assessments successfully, and her meticulous attention to detail helped me refine my writing and formatting skills for which I am indebted. I am so grateful that I could work with a skilled clinician such as Anna Fenton. I appreciate her expert clinical guidance, and her availability which meant I could reach out to her at any time, and be assured of a reply within minutes! I was also lucky to sit through Anna’s consultations with some of the patients in the current study, which was a truly valuable experience.

I am also sincerely grateful to Professor Chris Frampton for his kind and patient manner in explaining complex statistical concepts and for his insightful comments which encouraged me to think about this research from different perspectives. This work would not have been possible without the financial support of the University of Otago Doctoral Scholarship which I also wish to acknowledge.
During the years I spent at the Department of Psychological Medicine in Christchurch, I felt like a part of a family here, for which I wish to thank all my amazing colleagues, especially Andrea Bartram, Barbara Malthus, Bridget Kimber, Bridget Kinnersely, Geri McLeod, Janet Spittlehouse, Jenny Jordan, Judith Stone, Julia Martin, Michele Armstrong, Samantha Groves, and Wendy Mayes. Of these, Janet Spittlehouse and Jenny Jordan deserve special note for their great academic and emotional support.

I would like to express special appreciation to Wendy Sincock for her valuable help with formatting this thesis. Heartfelt gratitude to Lisa Andrews also for thorough formatting of this thesis in its later stage and for ensuring everything was trouble-free before submission.

I am thankful to Rebecca Phibbs, for all her expert help with Endnote and to Anna Young, Tim Young, Robert Densie, and Stephen Sharp for their I.T. and related assistance provided over the study period. My special thanks to Ruth Helms for being extremely supportive in all aspects of my PhD which significantly reduced my stress levels at critical times.

This research study would have been impossible without all the wonderful women who participated in the current study, giving their kindness, time and energy to this socially valuable project. I am sincerely grateful to all of them.

I am extremely fortunate to have a wonderful support system in the form of family and friends, both internationally, and here in Christchurch. Profound thanks to my mother, Mohini Vidwans, to my grand aunty, Vijaya Hari Darve, and my grandmother Vimal Vidwans for their unwavering love and support throughout my life, and especially during the last particularly challenging months of my PhD. I am deeply appreciative of my father, Anil Ram Sukhapure, for taking beautiful care of my Labrador, Pyaare, during my time here in New Zealand. Last but not least, I wish to thank all my friends here in Christchurch, with a special acknowledgement of Francis Johnson, for
always believing in me and encouraging me to trust my own being for guidance and strength.

Thank you all!
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Preface</td>
<td>v</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>vii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>x</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xiv</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xix</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xxi</td>
</tr>
<tr>
<td>1  THESIS OVERVIEW AND STRUCTURE</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Rationale and Aims</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Thesis Structure</td>
<td>4</td>
</tr>
<tr>
<td>2  DEPRESSION, COGNITIVE FUNCTION AND EMOTION PROCESSING</td>
<td>6</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>6</td>
</tr>
<tr>
<td>2.2 Major Depressive Disorder</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Prevalence</td>
<td>7</td>
</tr>
<tr>
<td>2.4 Risk Factors for Major Depressive Disorder</td>
<td>8</td>
</tr>
<tr>
<td>2.5 The Hypothalamic Pituitary Adrenal Axis</td>
<td>12</td>
</tr>
<tr>
<td>2.6 Associated Features of Major Depressive Disorder</td>
<td>13</td>
</tr>
<tr>
<td>2.7 Depression and Emotion Processing</td>
<td>21</td>
</tr>
<tr>
<td>2.8 Anxiety: Comorbidity and Prevalence of Anxiety in Depressed Samples</td>
<td>26</td>
</tr>
<tr>
<td>2.9 Treatment Strategies for Depression</td>
<td>28</td>
</tr>
<tr>
<td>2.10 Key Points</td>
<td>31</td>
</tr>
<tr>
<td>3  ANDROGENS IN FEMALES</td>
<td>33</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>33</td>
</tr>
<tr>
<td>3.2 Normal Androgen Function in Healthy Females</td>
<td>34</td>
</tr>
<tr>
<td>3.3 Types and Sources of Androgens</td>
<td>34</td>
</tr>
<tr>
<td>3.4 Neurobiology of Androgens</td>
<td>44</td>
</tr>
<tr>
<td>3.5 Androgen Disorders</td>
<td>50</td>
</tr>
<tr>
<td>3.6 Key Points</td>
<td>51</td>
</tr>
<tr>
<td>4  POLYCYSTIC OVARIAN SYNDROME</td>
<td>53</td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>53</td>
</tr>
<tr>
<td>4.2 Clinical Features</td>
<td>54</td>
</tr>
<tr>
<td>4.3 Diagnosis/Classification</td>
<td>59</td>
</tr>
<tr>
<td>4.4 Epidemiology</td>
<td>62</td>
</tr>
<tr>
<td>4.5 Risk Factors</td>
<td>64</td>
</tr>
<tr>
<td>4.6 Biological Mechanisms</td>
<td>67</td>
</tr>
</tbody>
</table>
4.7 Treatment .................................................................................................................. 70
4.8 Polycystic Ovarian Syndrome and Mood ................................................................. 75
4.9 Polycystic Ovarian Syndrome and Cognitive Function ............................................ 79
4.10 Conclusion and Implications .................................................................................... 85

5 ANDROGENS, DEPRESSION, ANXIETY, COGNITIVE FUNCTION AND
EMOTION PROCESSING IN WOMEN: A SYSTEMATIC REVIEW ..................... 87
5.1 Introduction .............................................................................................................. 87
5.2 Method ..................................................................................................................... 91
5.3 Results and Discussion ......................................................................................... 92
5.4 Androgen Levels and Emotion Processing .............................................................. 144
5.5 Conclusion ............................................................................................................ 149

6 METHODS ............................................................................................................... 151
6.1 Introduction ............................................................................................................ 151
6.2 Main Study Design ................................................................................................ 151
6.3 Participants ............................................................................................................. 152
6.4 Screening Instruments and Clinical Rating Scales .................................................. 155
6.5 Questionnaires or Additional Information from Participants ................................ 160
6.6 Cognitive Assessment ............................................................................................ 161
6.7 Description of Tasks in the Cognitive Testing Battery ............................................. 167
6.8 Endocrinological/Physical Assessment ................................................................ 182
6.9 Procedure .............................................................................................................. 186
6.10 Data Management ............................................................................................... 187
6.11 Statistical Analysis ............................................................................................... 189

7 STUDY 1: ASSOCIATIONS BETWEEN ANDROGEN LEVELS, AND MOOD,
ANXIETY, COGNITIVE FUNCTION AND EMOTION PROCESSING AT
BASELINE ............................................................................................................... 193
7.1 Introduction ............................................................................................................ 193
7.2 Recruitment Statistics ............................................................................................ 195
7.3 Participants ............................................................................................................. 195
7.4 Clinical Characteristics of the Polycystic Ovarian Syndrome Group ........................ 197
7.5 Factors that may Influence Cognitive Function ...................................................... 202
7.6 Analysis of Cognitive Data .................................................................................... 203
7.7 Verbal Learning and Memory Findings .................................................................. 204
7.8 Visuospatial Learning and Memory Findings ........................................................ 205
7.9 Psychomotor Speed Findings ................................................................................ 207
7.10 Attention and Executive Function Findings ........................................................ 207
7.11 Emotion Processing Findings .............................................................................. 208
7.12 Correlations between Testosterone Levels, Mood Ratings, Cognitive Variables
and Emotion Processing .............................................................................................. 211
7.13 Multivariate Regression Analysis ........................................................................ 223
7.14 Discussion .............................................................................................................. 225
7.15 Strengths of the Study .......................................................................................... 245
8 STUDY 2: MOOD, ANXIETY, COGNITIVE FUNCTION AND EMOTION PROCESSING IN POLYCYSTIC OVARIAN SYNDROME FOLLOWING ANTIANDROGEN TREATMENT .................................................. 252

8.1 Introduction .............................................................................. 252
8.2 Patients with Polycystic Ovary Syndrome ................................. 254
8.3 Change in Symptoms of Depression and Anxiety Over the Course of Treatment in the Polycystic Ovarian Syndrome (n = 42) and the Control (n = 50) Groups ............................................................................. 257
8.4 Change in Cognitive Function over the Course of Treatment for the Polycystic Ovarian Syndrome ................................................. 258
8.5 Change in Emotion Processing Over the Course of Treatment for Polycystic Ovarian Syndrome .......................................................... 262
8.6 Correlational Analysis ................................................................. 267
8.7 Discussion ................................................................................. 275
8.8 Strengths and Limitations of the Current Study ......................... 295
8.9 Conclusion ................................................................................. 299

9 SUMMARY AND CONCLUSIONS .................................................. 301

9.1 Study Overview ........................................................................ 301
9.2 Principal Findings ................................................................. 302
9.3 Implications of the Current Research ....................................... 311
9.4 Future Research .................................................................... 312
9.5 Conclusion ............................................................................ 314

REFERENCES .................................................................................. 315

APPENDICES.................................................................................. 381

Appendix A: Information sheet
Appendix B: Consent form
Appendix C: Flyers used to recruit Patients and Control Participants
Appendix D: Polycystic Ovarian Syndrome Questionnaire
Appendix E: Ethics Approval Letter
Appendix F: Voucher Payment Agreement Form
Appendix G: National Adults Reading Test
Appendix H: Demographic Questionnaire
Appendix I: Mini International Neuropsychiatric Interview
Appendix J: Hospital Anxiety and Depression Rating Scale and Quick Inventory of Depressive Symptomatology
Appendix K: Consonant Vowel Consonant Task
Appendix L: Visual Analogue Scale
Appendix M: Trail Making Test
Appendix N: Digit Span Test
Appendix O: Controlled Oral Word Association Test
Appendix P: Reading the Mind in the Eyes Test
Appendix Q: Research Request Form for Blood Samples
Appendix R: Order of Cognitive Tests in the Current Study
Appendix S: Tables Showing Correlations between Testosterone Levels and Mood, Anxiety and Cognitive Function (Raw and Adjusted Scores: Before and After Controlling For NART)
Appendix T: Scatterplot Showing Associations between Body Mass Index and Hospital Anxiety and Depression Scale- Depression Subscale Score In the Polycystic Ovarian Syndrome (N = 53) and Control (N = 50) Groups at Baseline.
Appendix U: Correlations Between Ferriman-Gallwey (FG) Score and Testosterone Levels, Mood and Anxiety in Polycystic Ovarian Syndrome (N = 27)
Appendix V: Box Plot Showing an Overlap between Free Androgen Index Levels across Polycystic Ovarian Syndrome (N = 53) and Control (N = 50) Groups.
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3.1</td>
<td>Androgen Production in Women</td>
<td>39</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Common Symptoms of Polycystic Ovarian Syndrome</td>
<td>58</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Diagnostic Criteria for Polycystic Ovarian Syndrome</td>
<td>61</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Meta-Analytical Findings Related to Depression and Anxiety in Females with Polycystic Ovarian Syndrome</td>
<td>80</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>Studies Examining the Relationship between Androgen Levels, Mood and Anxiety in Females with Polycystic Ovarian Syndrome</td>
<td>95</td>
</tr>
<tr>
<td>Table 5.2</td>
<td>Studies Examining the Relationship between Androgen Levels, Mood and Anxiety in Reproductive-aged Females without Polycystic Ovarian Syndrome</td>
<td>100</td>
</tr>
<tr>
<td>Table 5.3</td>
<td>Cross-sectional Studies Examining the Relationship between Androgen Levels, Mood, Anxiety, Cognitive Function and Emotion Processing in Reproductive-aged Females with and without Polycystic Ovarian Syndrome</td>
<td>103</td>
</tr>
<tr>
<td>Table 5.4</td>
<td>Interventional Studies Examining the Relationship between Androgen Levels, Mood, Anxiety, Cognitive Function and Emotion Processing in Reproductive-aged Females with and without Polycystic Ovarian Syndrome</td>
<td>110</td>
</tr>
<tr>
<td>Table 6.1</td>
<td>Order of Test Administration in the Cognitive Testing Battery</td>
<td>166</td>
</tr>
<tr>
<td>Table 6.2</td>
<td>List A and List B of Nonsense Syllables Included in the Consonant-Vowel-Consonant Task at Baseline and Follow-up to Assess Verbal Learning and Memory</td>
<td>170</td>
</tr>
<tr>
<td>Table 7.1</td>
<td>Means (SD) or Percentages for Demographic Characteristics in Polycystic Ovarian Syndrome ($n = 50$) and Control ($n = 53$) groups</td>
<td>197</td>
</tr>
<tr>
<td>Table 7.2</td>
<td>Means (SD) of Raw Scores of Androgen Levels and Other Hormonal Levels in the Polycystic Ovarian Syndrome and Control Groups</td>
<td>198</td>
</tr>
<tr>
<td>Table 7.3</td>
<td>Means (SD) and Ranges for Clinical Characteristics in the Polycystic Ovarian Syndrome ($n = 50$) and Control ($n = 53$) Groups</td>
<td>199</td>
</tr>
</tbody>
</table>
Table 7.4  Presence of Current Axis I Disorders in the Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups ........................................ 200

Table 7.5  Anti-androgen and Other Hormonal Medication used by the Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups at the Baseline Assessment .......................................................... 201

Table 7.6  State Anxiety in the Polycystic Ovarian Syndrome Group \((n = 50)\) Compared with the Control \((n = 53)\) Group at the Baseline Assessment ...... 202

Table 7.7  Adjusted Means (SD) and Effect Sizes on the Consonant-Vowel-Consonant Task in the Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups .................................................................................. 204

Table 7.8  Adjusted Mean Total Errors (SD) and Effect Sizes for the Groton Maze Learning Test in the Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups ................................................................. 206

Table 7.9  Means (SD) and Effect Sizes for Psychomotor Speed Measures in the Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups ........ 207

Table 7.10  Means (SD and SEM) and Effect Sizes on Attention and Executive Function Variables in Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups ................................................................. 208

Table 7.11  Means (SD) of Facial Expression Recognition (Accuracy and Neutral Misinterpretation) Scores, and Effect Sizes in the Polycystic Ovarian Syndrome \((n = 50)\) and Control \((n = 53)\) Groups ........................................................................... 210

Table 7.12  Correlations between Testosterone Variables and Depression and Anxiety Variables across the Entire Sample \((n = 103)\) after Controlling for Verbal IQ (NART) ................................................................................. 213

Table 7.13  Correlations between Testosterone Variables and Consonant Vowel Consonant Task (CVC) Variables across the Entire Sample \((n = 103)\) after Controlling for Verbal IQ (NART) ............................................................................. 214

Table 7.14  Correlations between Testosterone Variables and Groton Maze Learning Test Variables across the Entire Sample \((n = 103)\) after Controlling for Verbal IQ (NART) ................................................................................. 215

Table 7.15  Correlations between Testosterone Variables and Psychomotor Speed Variables across the Entire Sample \((n = 103)\) after Controlling for Verbal IQ (NART) ................................................................................. 215

Table 7.16  Correlations between Testosterone Variables and Attention and Executive Function Variables across the Entire Sample \((n = 103)\) after Controlling for Verbal IQ (NART) ................................................................. 216
Table 7.17  Correlations between Testosterone Variables and Facial Expression Recognition Variables (Recognition Accuracy) using Mood (Hospital Anxiety and Depression Rating Scale - Depression Subscale) as a Covariate across the Entire Sample (n = 103).......................... 218

Table 7.18  Correlations between Testosterone Variables and Facial Expression Recognition Variables (Reaction Time) using Mood (Hospital Anxiety and Depression Rating Scale- Depression Subscale) as a Covariate across the Entire Sample (n = 103)................................................................. 219

Table 7.19  Correlations between Testosterone levels and Facial Expression Recognition Variables (Neutral Misinterpretation Bias) using Mood (Hospital Anxiety and Depression Rating Scale - Depression Subscale) as a Covariate across the Entire Sample (n = 103)................................................................. 220

Table 7.20  Correlations between Mood and Anxiety Variables and Consonant-Vowel-Consonant Task Variables across the Entire Sample (n = 103).... 221

Table 7.21  Correlations between Mood and Anxiety Variables and Groton Maze Learning Test Variables across the Entire Sample (n = 103)......................... 222

Table 7.22  Correlations between Mood and Anxiety Variables and Psychomotor Variables across the Entire Sample (n = 103)......................................................... 223

Table 7.23  Correlations between Mood and Anxiety Variables and Attention and Executive Function Variables across the Entire Sample (n = 103)............. 224

Table 7.24  Summarising Correlations between Testosterone Variables and Mood and Anxiety Variables and Cognitive Function and Emotion Processing Variables across the Entire Sample (n = 103)................................................................. 245

Table 8.1  Means (SD) for Demographic Variables in Study Completers (n = 42) Versus Non-Completers (n = 8) in the Polycystic Ovarian Syndrome Group (n = 50)........................................................................................................... 255

Table 8.2  Hormonal Treatment Profile of Patients with Polycystic Ovarian Syndrome at Follow-up ................................................................................................. 256

Table 8.3  Change in Symptoms of Depression and Anxiety Over the Course of Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups ............................................................................................................. 257

Table 8.4  Means (SD) and Effect Sizes of Change in Consonant-Vowel-Consonant Task Variables following Anti-androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups ......................... 259
Table 8.5  Means (SD) and Effect Sizes of Change in Groton Maze Learning Test Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups ............................................. 260

Table 8.6  Means (SD) and Effect Sizes of Change in Psychomotor Speed Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups .............................................................. 261

Table 8.7  Means (SD) and Effect Sizes of Change in Attention and Executive Function Variables in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups .............................................................. 263

Table 8.8  Means (SD) and Effect Sizes of Change in Recognition Accuracy on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups .............................................................. 264

Table 8.9  Means (SD) and Effect Sizes of Change in Reaction Time on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups .............................................................. 265

Table 8.10 Means (SD) and Effect Sizes of Change in Neutral Misinterpretation Bias on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups .............................................................. 266

Table 8.11 Correlations between Change in Mood and Anxiety Variables and Change in Consonant-Vowel-Consonant Task Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) ................. 268

Table 8.12 Correlations between Change in Mood and Anxiety Variables and Change in Groton Maze Learning Test Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) ................. 269

Table 8.13 Correlations between Change in Mood and Anxiety Variables and Change in Psychomotor Speed Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) .................................................. 270

Table 8.14 Correlations between Change in Mood and Anxiety Variables and Change in Attention and Executive Function Variables following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) ................. 271

Table 8.15 Correlations between Change in Mood and Anxiety Variables and Accuracy on the Facial Expression Recognition Task Following Anti-Androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) ............... 273

Table 8.16 Correlations between Change in Mood and Anxiety Variables and Mean Reaction Time on the Facial Expression Recognition Task Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) ... 273
Table 8.17 Correlations between Change in Mood and Anxiety Variables and Neutral Misinterpretation Bias on the Facial Expression Recognition Task Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42) .......................................................... 274

Table 9.1 Comparison of Current Findings Related to Mood and Anxiety in Females with Polycystic Ovarian Syndrome with Previous Studies .......... 307

Table 9.2 Comparison of Current Findings Related to Cognitive Function and Emotion Processing in Females with Previous Studies in Females with and without Polycystic Ovarian Syndrome ................................................. 308
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Major Depressive Disorder rates by age and sex</td>
<td>10</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Steroidogenesis in the ovaries, adrenal glands and peripheral tissues of androgens in females</td>
<td>40</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Proposed pathophysiology of Polycystic Ovarian Syndrome</td>
<td>69</td>
</tr>
<tr>
<td>Figure 6.1</td>
<td>Visual Analogue Scale used to measure state anxiety at three time-points during the cognitive testing</td>
<td>162</td>
</tr>
<tr>
<td>Figure 6.2</td>
<td>Groton Maze Learning Test initial screen</td>
<td>171</td>
</tr>
<tr>
<td>Figure 6.3</td>
<td>Six basic facial emotions; anger, disgust, fear, happiness, sadness and a neutral expression</td>
<td>179</td>
</tr>
<tr>
<td>Figure 6.4</td>
<td>An example item from the Reading the Mind in the Eyes Test</td>
<td>182</td>
</tr>
<tr>
<td>Figure 6.5</td>
<td>Timeline of Study Procedure</td>
<td>188</td>
</tr>
<tr>
<td>Figure 6.6</td>
<td>Overview of Study Design</td>
<td>189</td>
</tr>
<tr>
<td>Figure 7.1</td>
<td>Mean (+SD) number of words recalled on the five learning trials and delayed recall trial of the Consonant Vowel Consonant Task in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups</td>
<td>205</td>
</tr>
<tr>
<td>Figure 7.2</td>
<td>Mean (+SD) Total number of errors on the Groton Maze Learning Test (GMLT) over the five learning trials (1-5) and the delay trial in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups</td>
<td>206</td>
</tr>
<tr>
<td>Figure 7.3</td>
<td>Mean (+SD) Recognition accuracy for the five facial expressions of emotional and neutral expressions on the Facial Expression Recognition Task in Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups</td>
<td>209</td>
</tr>
<tr>
<td>Figure 7.4</td>
<td>Mean (+SD) Misinterpretation of neutral faces for the five facial emotional expressions on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups</td>
<td>211</td>
</tr>
<tr>
<td>Figure 8.1</td>
<td>Change in scores displayed as mean (+SD) number of words recalled on Trial 1, 5, 1-5 (total learning) and Delayed Recall Trial of the Consonant Vowel Consonant Task variables in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time</td>
<td>259</td>
</tr>
</tbody>
</table>
Figure 8.2  Change in scores displayed as mean (± SD) total number of errors on the Groton Maze Learning Test variables in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) groups over time .......................... 260

Figure 8.3  Change in scores displayed as mean (+SD) recognition accuracy for the five facial expressions of emotion and neutral expressions on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time ........................................... 264

Figure 8.4  Change in scores displayed as mean (+SD) misinterpretation of neutral faces for the five facial expressions of emotion on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time ................................................... 266
List of Abbreviations

ACC ................... Anterior Cingulate Cortex
ACTH ................. Adrenocorticotropic Hormone
A-dione ............ Androstenedione
A-diol ............... Androstenediol
AE-PCOS .......... Androgen Excess and PCOS Society
AMH .................. Anti-Mullerian Hormone
ANOVA .............. Analysis of Variance
ANCOVA .......... Analysis of Covariance
ASD .................. Autistic Spectrum Disorder
BBB ................. Blood Brain Barrier
BDI .................. Beck Depression Inventory
BMI ................. Body Mass Index
BOLD .............. Blood-oxygen-level Dependent
BPD .................. Borderline Personality Disorder
CAH .................. Congenital Adrenal Hyperplasia
CBT .................. Cognitive Behavioural Therapy
CDHB .............. Canterbury District health Board
COWAT .......... Controlled Oral Word Association Test
CPA .................. Cyproterone Acetate
CRF .................. Corticotrophin Releasing Factor
CVC Task .......... Consonant Vowel Consonant Task
DHEA .............. Dehydroepiandrosterone
DHEAS .......... Dehydroepiandrosterone Sulphate
DHT ............... Dihydrotestosterone
DSI .................. DeRogatis Symptom Inventory
DSM .................. Diagnostic and Statistical Manual of Mental Disorders
FAI ................. Free Androgen Index
FER .................. Facial Expression Recognition
fMRI ............... Functional Magnetic Resonance Imaging
Free T .......... Free Testosterone
FSH ............... Follicle Stimulating Hormone
GAD ............. Generalised Anxiety Disorder
GMLT .......... Groton Maze Learning Test
HADS .......... Hospital Anxiety and Depression Rating Scale
HAM-D ........ Hamilton Depression Rating Scale
HIV .............. Human Immunodeficiency Virus
HPA .......... Hypothalamic-Pituitary-Adrenal
IFG ............ Inferior Frontal Gyrus
IGF-1 .......... Insulin Growth Factor-1
IPT ............. Interpersonal Therapy
LH .............. Luteinising Hormone
MADRS ........ Montgomery-Asberg Depression Rating Scale
MAOI .......... Monoamine Oxidase Inhibitors
MDD .......... Major Depressive Disorder
MINI .......... Mini International Neuropsychiatric Interview
MMPI .......... Minnesota Multiphasic Personality Inventory
MRT ............ Mental Rotation Test
NART .......... National Adults Reading Test
NDRI ............ Noradrenaline-dopamine Reuptake Inhibitors
NIH ............ National Institutes of Health
OCP ............ Oral Contraceptive Pill
PASAT .......... Paced Auditory Serial Addition Task
PCOM .......... Polycystic Ovarian Morphology
PCOS .......... Polycystic Ovarian Syndrome
POMS .......... Profile of Mood States
QoL ............ Quality of Life
QIDS .......... Quick Inventory of Depressive Symptomatology
RAVLT.........Rey Auditory-Verbal Learning Task
RMET.........Reading the Mind in the Eyes Test
SAD............Social Anxiety Disorder
SCID............Structured Clinical Interview for DSM-IV
SHBG............Sex Hormone Binding Globulin
SNRI..........Serotonin Noradrenaline Reuptake Inhibitor
SPSS..........Statistical Packages for Social Sciences
SSRI..........Selective Serotonin Reuptake Inhibitor
STAI..........State Trait Anxiety Inventory
TCA..........Tricyclic Antidepressants
TCT..........Timed Chase Test
TMT..........Trail Making Test
Total T........Total Testosterone
VAS..........Visual Analogue Scale
WAIS..........Wechsler Adult Intelligence Scale
WHO..........World Health Organisation
ZARS..........Zung Anxiety Rating Scale
2D:4D ratio....Digit Length Ratio
11OHA..........11β hydroxyandrostenedione
CHAPTER 1

THESIS OVERVIEW AND STRUCTURE

1.1 BACKGROUND

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in reproductive-aged females (Azziz, Woods, et al., 2004; Franks, 1995; McGowan, 2011), affecting up to five to 22% of females depending upon the diagnostic criteria used (Azziz et al., 2016; Farquhar, Birdsell, Manning, Mitchell, & France, 1994; Goodarzi & Azziz, 2006; Mueller, Grissom, & Dohanich, 2014; Sirmans & Pate, 2014; Teede, Deeks, & Moran, 2010). It is well-established that the primary hormonal abnormality in PCOS is androgen excess (Azziz, Carmina, Dewailly, Diamanti-Kandarakis, Escobar-Morreale, Futterweit, Janssen, Legro, Norman, & Taylor, 2006), mainly Free Testosterone excess (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Although a greater rate of psychiatric illnesses, such as depression and anxiety disorders, have been found in PCOS (Cooney & Dokras, 2017; Cooney, Lee, Sammel, & Dokras, 2017; Dokras, Clifton, Futterweit, & Wild, 2011, 2012), very little research has directly examined the relationship between abnormal androgen levels and symptoms of depression and anxiety in females with or without PCOS.

Major depressive disorder (MDD) is a common and debilitating condition which affects more than 322 million individuals of all ages worldwide (World Health Organisation, 2017). It is projected to be one of the three leading causes of disease burden in 2030 (Mathers & Loncar, 2006) and has an enormous cost of illness and impairment in individuals’ functioning (Johnson, Weissman, & Klerman, 1992; Kessler & Bromet, 2013; Spijker et al., 2004). Females are twice as likely to suffer from mood and anxiety disorders as males, and symptoms of mood and
anxiety are often of greater severity in females (Karger, 2014; Noble, 2005; Weissman et al., 1993; Weissman & Olfson, 1995). Research has consistently found impairment in cognitive function, including deficits in memory, attention and executive function, and emotion processing in individuals with MDD (Douglas & Porter, 2009; Douglas, Porter, Knight, & Maruff, 2011; Hsu, Young-Wolff, Kendler, Halberstadt, & Prescott, 2014; Porter, Bourke, & Gallagher, 2007; Porter, Gallagher, Thompson, & Young, 2003; Porter, Robinson, Malhi, & Gallagher, 2015; Rock, Roiser, Riedel, & Blackwell, 2013; Zalkanis, Leach, & Kaplan, 1998). However, current first-line pharmacological treatments for MDD have limited efficacy and unwanted side-effects (Arroll et al., 2009) and do little to improve cognitive impairment found in MDD (Airaksinen, Wahlin, Larsson, & Forsell, 2006; Porter et al., 2016; Shilyansky et al., 2016). Thus, there are likely to be many females in the general population who have depression and cognitive impairment as a result of untreated androgen abnormalities, however, the relationship between androgen levels, mood and associated cognitive function has not been investigated directly. Normalising androgens may be a more effective and logical treatment strategy than antidepressant treatment. This thesis presents data from a longitudinal study which examined in detail associations between androgen levels and symptoms of depression, cognitive functioning and emotion processing in females of reproductive age, with and without PCOS, before and after 12 weeks of anti-androgen treatment in the PCOS group.

1.2 RATIONALE AND AIMS

The current study aimed to investigate the relationship between androgen levels and mood, cognitive function and emotion processing, and for this purpose included reproductive-aged females with PCOS, with abnormally elevated levels of testosterone, as well as reproductive-aged non-PCOS control females. Females newly referred to a gynaecological-endocrine clinic were assessed and a treatment plan was put in place by the treating Gynaecological-Endocrinologist.
Standard clinical medication included anti-androgen agents to either decrease or block androgen levels. Before commencement of anti-androgen treatment, the baseline assessment including mood and cognitive assessment was carried out, as one of the main goals of the current study was to examine the associations between androgen levels, symptoms of mood, cognitive function and emotion processing at baseline. This also helped to determine whether treating abnormalities in the androgen system in the patient group affected symptoms of mood, anxiety and cognitive function. Additionally, an investigation was carried out to examine whether a change in mood following treatment in the PCOS group was associated with a change in cognitive function and emotion processing variables. This is the first study to simultaneously examine the association between androgen levels and mood, cognitive function and emotion processing in reproductive-aged females with and without PCOS, and to examine the association between change in mood and change in cognitive function and emotion processing variables in the PCOS group following anti-androgen treatment. Findings from the current thesis may help facilitate a healthy collaboration between the fields of psychiatry and endocrinology.

For the main purpose of determining whether a relationship exists between androgen levels, depressive symptoms and cognitive function, the main tests consisted of:

1. Clinical measures – clinician-administered and self-report mood and anxiety rating scales,
2. Cognitive measures - assessment of domains including verbal and visuospatial learning and memory, attention and executive function, psychomotor speed, and emotion processing,
3. Medical and endocrinological measures - laboratory assessment included androgen levels and other important hormone levels from blood samples, along with an assessment of the physical symptoms involved in PCOS including hirsutism, acne and alopecia.
1.3 THESIS STRUCTURE

The current thesis has the following structure:

Chapter Two presents an overview of MDD, with a discussion of the heterogeneous nature of depression, and the key symptoms and causes involved. Associated features of cognitive impairment, impairment in emotion processing and comorbid anxiety, along with treatment strategies, are also discussed.

Chapter Three presents an overview of the main types and sources of androgens and normal androgen activity in healthy females. The second part of the chapter focuses on the neurobiology of androgens along with an overview of disorders related to androgen-excess in females.

Chapter Four provides a detailed discussion of PCOS, with an overview of the clinical features involved and the diagnostic criteria and classification. The second part of the chapter discusses its epidemiology, risk factors, biological mechanisms and the treatment for PCOS. Following this, findings related to mood, cognitive function and emotion processing in females with PCOS are reviewed.

Chapter Five presents the findings of a systematic literature review of studies assessing mood, anxiety, cognitive function and emotion processing in relation to androgen levels in females of reproductive age with and without PCOS.

Chapter Six describes the methodology used in subsequent chapters.

Chapter Seven presents findings from baseline analyses examining associations between androgen levels, mood and anxiety symptoms, and performance on measures of cognitive
function, and emotion processing in females with PCOS compared with non-PCOS control females, and in the entire group as a whole, followed by a discussion of the results.

Chapter Eight presents longitudinal mood and cognitive findings over the course of the 12-week anti-androgen treatment in females with PCOS compared with non-PCOS control females receiving no treatment, followed by a discussion of the results.

Chapter Nine presents an overall summary of findings, their implications and directions for future research.
CHAPTER 2

DEPRESSION, COGNITIVE FUNCTION AND EMOTION
PROCESSING

2.1 INTRODUCTION

This chapter presents an overview of major depressive disorder (MDD) with a discussion of the heterogeneous nature of MDD, the key symptoms and causes involved, and the reasons behind gender differences in mood and anxiety disorders. Following this, associated features of cognitive impairment, emotion processing impairment, and comorbid anxiety are described. Lastly, treatment strategies for depression are discussed.

2.2 MAJOR DEPRESSIVE DISORDER

2.2.1 Key symptoms

Major depressive disorder is widely recognised as a common and debilitating condition, characterised by pervasive low mood or sadness. Depression is a heterogeneous disorder with a variable course. It is distinct from fluctuations in mood in healthy individuals and is associated with diminished quality of life, impaired social function, medical morbidity and mortality (Kessler, Berglund, Demler, & et al., 2003; Kessler & Bromet, 2013; Noble, 2005). The Diagnostic and Statistical Manual of Mental Disorders - 5th edition (DSM-5) states that the diagnosis of MDD requires the presence of depressive symptoms involving a distinct change in mood characterised by irritability and/or sadness, accompanied by several features including loss of interest in otherwise pleasurable activities, sleep disturbance, changes in appetite and
weight, feelings of worthlessness or hopelessness, lack of focus and concentration, and suicidal ideation. These symptoms must last for a minimum of two weeks and cause clinically significant distress or role impairment in social and occupational functioning or in other areas of life important to the individual. A major depressive episode may occur in the context of MDD (involving exclusively depressed mood) or bipolar disorder (in which hypomanic or manic episodes may be present at other times).

It is understood that the symptoms of depression occur on a continuum of severity, with greater severity of symptoms being positively associated with higher psychiatric comorbidity (Kessing, 2007; Lewinsohn, Solomon, Seeley, & Zeiss, 2000). Since the clinical picture of depression is heterogeneous and involves variability in the degree of severity of symptoms (Snaith, 1987), it is important to acknowledge the spectrum on which this condition lies, ranging from mild to severe depression. Although a larger proportion of depression-related literature focuses on the diagnosable clinical state of depression, the overall prevalence rates of individuals with symptoms of depression is still high (Angst & Merikangas, 1997). In the current thesis, the term ‘depression’ will be used unless referring to studies that talk specifically about MDD as a diagnostic classification.

2.3 PREVALENCE

2.3.1 Global burden of depression and prevalence in New Zealand, and internationally

The World Health Organisation (WHO) reports that more than 322 million people of all ages live with MDD globally (at one time-point), with more females (5.1%) affected compared with males (3.6%) (World Health Organisation, 2017). The total estimated number of individuals with depression increased by 18.4% between 2005 and 2015. Currently, MDD is the leading cause of disease-related disability worldwide (Flint & Kendler, 2014) and has an enormous cost
of illness and impairment in individuals’ functioning (Cassano & Fava, 2002; Johnson et al., 1992; Kessler & Bromet, 2013; Spijker et al., 2004). Consequently, MDD is a priority condition for WHO and is covered by their Mental Health Gap Action Programme (World Health Organisation, 2008). In New Zealand, the burden of MDD is high. The New Zealand Mental Health Survey (Te Rau Hinengaro) reported a high prevalence of MDD in adults (14.3%), with significantly higher rates in females closer to reproductive age (35 to 44 years) (17.9%) compared with males (10.4%) (Oakley-Browne, Wells, & Scott, 2006).

Similar findings have been documented worldwide, with MDD affecting twice as many females as males in adulthood (Karger, 2014; Kessler, 2003; Kessler et al., 1994; Noble, 2005; Piccinelli & Wilkinson, 2000; Weissman et al., 1993; Weissman & Olfson, 1995). The National Institutes of Mental Health (United States) report that females are 70% more likely to suffer from depression compared with males. This gender difference in prevalence of depression has been consistently found despite variability in ethnic groups and cultures (Waraich, Goldner, Somers, & Hsu, 2004).

2.4 RISK FACTORS FOR MAJOR DEPRESSIVE DISORDER

The aetiology of MDD is still not fully understood due to its heterogeneity. However, multiple theories have been postulated to explain the development and progression of MDD, including genetic (Flint & Kendler, 2014; Kendler & Karkowski-Shuman, 1997; Kendler et al., 2010; Silberg et al., 1999), neurobiological (neurotransmitter systems including noradrenaline and serotonin) (Delgado & Moreno, 2000; Malhi, Parker, & Greenwood, 2005; Nestler et al., 2002; Ressler & Nemeroff, 2000) and neuroendocrinological theories (Belmaker & Agam, 2008; Checkley, 1996; Drevets, Price, & Furey, 2008), together with psychological and social explanations (Beck, 1979; Bonde, 2008; Hagerty & Williams, 1999; Peterson & Seligman, 1984) and an interaction between models has also been suggested (Beck, 2008; Nemeroff &
Vale, 2005). Other risk factors may include adverse childhood experiences (D. Chapman et al., 2004), and life events including trauma or stress (Kendler & Karkowski-Shuman, 1997). The hormonal causes of depression are more relevant to the current thesis, and therefore, will be discussed in detail.

2.4.1 **Neuroendocrinological risk factors for major depressive disorder**

2.4.1.1 **Sex hormones**

There is a higher prevalence and greater symptom severity of MDD in females compared with males (see Figure 2.1) (Bebbington, 1996; Kessler, 2003; Noble, 2005; Nolen-Hoeksema & Hilt, 2013; Piccinelli & Wilkinson, 2000). Additionally, an earlier age of onset, atypical features and dysthymia (a chronic and persistent form of depressive disorder) are more frequently observed in females compared with males (Angst & Merikangas, 1997). The observed gender difference in the prevalence of symptoms has piqued research interest in the investigation of the relationship between sex hormones and mood in individuals with MDD (Baischer, Koinig, Hartmann, Huber, & Langer, 1995; Bromberger, Schott, & Kravitz, 2010; Dokras, 2012; Dokras et al., 2011; Rohr, 2002; Yonkers, 2003). For at least some females, depression may have hormonal underpinnings, although the exact hormonal determinants have not been entirely established (Piccinelli & Wilkinson, 2000).
Figure 2.1  
Major depression rates by age and sex  
(Steiner, 2009; Weissman, Bruce, Leaf, Florio, & Holzer, 1991).

2.4.1.2 Female-specific sex hormonal factors

Studies have investigated oestrogen (primary female sex hormone) and progesterone (female hormone important in maintaining pregnancy) levels in relation to mood in females. Fluctuating levels of sex hormones (oestrogen, progesterone and testosterone) through variations in reproductive phases including puberty, menarche, pregnancy and menopause can be accompanied by conditions affecting mood, such as premenstrual syndrome, postpartum and postmenopausal depression (Lawrie, Herxheimer, & Dalton, 2000; Steiner, Dunn, & Born, 2003; Yonkers, 2003; Zsido, Villringer, & Sacher, 2017). Such patterns indicate an effect of sex hormones on mood. Furthermore, there is a marked increase in the incidence of depression in females from puberty onwards, which declines following menopause. Steiner et al. (2009) reported that the constant fluctuation of oestrogen and progesterone during various reproductive stages in females may contribute to frequently altered sensitivity in neurotransmitter systems or suppression of the Hypothalamic-Pituitary-Adrenal (HPA) axis (see page 13), further leading
to the development of depression. Although progesterone has been frequently examined in females with postnatal depression, evidence regarding this relationship is still weak and requires more investigation (Granger & Underwood, 2001; Klier et al., 2007).

On the other hand, there is relatively consistent evidence regarding an inverse relationship between oestrogen levels and an increased incidence of depression (Slowik, Lammerding, Hoffmann, & Beyer, 2018; Wharton, Gleason, Sandra, Carlsson, & Asthana, 2012; Young, Midgley, Carlson, & Brown, 2000; Zheleznova, Medvedev, & Kalinin, 2013). Oestrogen is thought to act on the hippocampus in a similar manner to antidepressants that selectively inhibit serotonin (one of the main neurotransmitters involved in depression) reuptake. Lower oestrogen levels have been associated with an impaired central adrenergic function (including disrupted norepinephrine and epinephrine function), which has been related to depression (Klaiber, Broverman, Vogel, & Kobayashi, 1979). Studies have found some benefit in oestrogen supplementation as an adjunct to the pharmacological treatment of MDD and symptoms of depression in samples including females with postnatal depression or perimenopausal and postmenopausal females (de Novaes Soares, Almeida, Joffe, & Cohen, 2001; Halbreich & Kahn, 2001; Klaiber et al., 1979; P. J. Schmidt et al., 2000; Zweifel & O'Brien, 1997).

2.4.1.3 Androgens

Although androgens are primarily male sex hormones, they play an important physiological role in both males and females. As the current thesis is focused on the relationship between androgen levels and mood, Chapters 3 and 4 involve a thorough description of androgens, and a discussion of conditions (mainly Polycystic Ovarian Syndrome (PCOS), involving chronically elevated levels of androgens) that are associated with increased rates of depression. Chapter 5 presents a detailed review of studies investigating associations between androgens and mood in reproductive-aged females.
2.5 THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The Hypothalamic Pituitary Adrenal (HPA) axis is a feedback system which plays an important role in human adaptation to physical and psychological stress. In a normal individual, following a perceived stressor, the HPA axis activity regulates secretion of the adrenocorticotropic hormone-releasing hormone (CRH) and arginine-vasopressin from the hypothalamus, which stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary. Adrenocorticotropic hormone then acts on the adrenal cortex to produce cortisol, which is a glucocorticoid stress hormone. Cortisol has crucial effects on multiple tissues in the brain and body (Burke, Davis, Otte, & Mohr, 2005). Cortisol binds to its receptors in the hypothalamus and inhibits secretion of CRF and ACTH, respectively, both during and after the stressful event has receded. This, in turn, leads to lowered cortisol secretion by the adrenal cortex, known as the negative feedback mechanism of the HPA axis. This inhibitory feedback mechanism works at the level of the hypothalamus, pituitary gland, and hippocampus to restore homeostasis through self-regulation.

A relatively consistent neuroendocrine abnormality found in depression is hyperactivity of the HPA axis (Pariante & Lightman, 2008; Porter & Gallagher, 2006; Schatzberg et al., 2013). Evidence suggests that excess cortisol production or hypercortisolism as a result of a dysfunctional HPA axis is associated with symptoms of depression (Burke et al., 2005; De Kloet, 2004; Peeters, Nicholson, & Berkhof, 2003), and that cortisol suppression helps to improve low mood (Murphy, 1991). Dysfunction in the HPA axis may be associated with cognitive impairment (see section 2.6.1) (Porter & Gallagher, 2006). Some studies have suggested that the HPA axis is more reactive to stress in females compared with males (Nolen-Hoeksema & Hilt, 2013; Oldehinkel & Bouma, 2011) and that ovarian hormones modulate regulation of the HPA axis, which contributes to HPA dysfunction (Young & Korszun, 1999). The HPA axis not only regulates physiological mechanisms but has a significant effect on the
brain, affecting mood and cognition (Holsboer, 2000; Keller et al., 2016; Porter & Gallagher, 2006; S. Watson & Mackin, 2006)

Sex hormones may play a mediating role in HPA axis function (Da Silva, 1999; Handa, Burgess, Kerr, & O'Keefe, 1994). Of interest to the current thesis is the hypothesis that since the adrenal glands are an important source of androgens, including testosterone, androstenedione (60 to 70%), and precursor androgen dehydroepiandrosterone (DHEA), any disruptions in the HPA axis may ultimately affect androgen synthesis. In certain sub-groups of mood disorders, such as bipolar disorder and MDD with psychotic features, HPA axis abnormalities are more pronounced. There is also evidence of a disrupted HPA axis in individuals with severe or melancholic MDD (Cowen, 2009; Keller et al., 2016; Pariante & Lightman, 2008). However, it is beyond the scope of the current thesis to discuss in detail the clinical features of depression which are associated with the HPA axis abnormalities, or the nature of melancholic depression and whether this is a distinct and definable subtype. Studies have shown antidepressant medication may help resolve abnormalities in the HPA system (Jensen et al., 1999; Mason & Pariante, 2006).

2.6 ASSOCIATED FEATURES OF MAJOR DEPRESSIVE DISORDER

Major depressive disorder is a heterogeneous disorder with associated features including impairment in cognitive function and in emotion processing, which has a major impact on individuals’ overall quality of life (Porter et al., 2007; Porter et al., 2015; Rock et al., 2013). This section will discuss the nature of cognitive impairment in depression and describe key domains associated with symptoms of depression, both during depressive episodes and longitudinally.
2.6.1 Cognitive impairment in depression

Cognitive impairment is well-established as a core feature of depression (Douglas & Porter, 2009; Douglas et al., 2011; Hsu et al., 2014; McDermott & Ebmeier, 2009; Porter et al., 2007; Porter et al., 2003; Rock et al., 2013; Zalkanis et al., 1998). Studies have linked poor response to treatment with cognitive deficits, including poorer attention and executive function, and psychomotor speed (Dunkin et al., 2000; Etkin et al., 2014; Gudayol-Ferré et al., 2012; B. Taylor et al., 2006; Withall, Harris, & Cumming, 2009), however, data is still inconclusive in adult samples. Until now, no conventional antidepressant treatment has been thoroughly examined to show pro-cognitive benefits in individuals with MDD (McIntyre et al., 2013). Cognitive impairment contributes to substantial problems in occupational and interpersonal functioning and such residual impairment in psychosocial functions may continue after remission from depression (Hasselbalch, Knorr, & Kessing, 2011; Jaeger, Berns, Uzelac, & Davis-Conway, 2006; Kennedy, Foy, Sherazi, McDonough, & McKeon, 2007).

The following sections will provide an overview of the main types of cognitive domains and key areas of impairment found in depression.

2.6.2 Cognitive domains impaired in depression

Categorising cognitive functions into specific domains is complex since most cognitive tasks assess multiple cognitive processes, however, most tasks are related to a specific domain more completely than other domains. Therefore, the following categories are generally recognised as major cognitive domains.
2.6.2.1 Attention

The construct of attention extends back to the start of experimental psychology, however, to date, no universally accepted definition of attention exists (Lezak, 2004). Attention refers to multiple different processes involved in how the individual becomes receptive to stimuli. Some cognitive tasks require higher levels of attention compared with other cognitive domains, and are thus, known as measures of attention. Four aspects of attention are clinically relevant: i) focused or selective attention- this is the most studied type of attention, and refers to the capacity to prioritise one or two salient stimuli while at the same time suppressing other competing stimuli (concentration), ii) sustained attention - the ability to keep continuous focus on a particular task, over a period of time (Langner & Eickhoff, 2013), iii) divided attention - the capacity to focus and respond to several stimuli simultaneously, and iv) alternating attention - this involves flexibility to mentally switch between different tasks, as required. Traditional tests of attention are usually global measures of attention rather than tasks that specifically measure one of these components. For example, the Paced Auditory Serial Addition Task (PASAT) (Diehr, Heaton, Miller, & Grant, 1998) and Rapid Visual Information Processing (Coull, Frith, Frackowiak, & Grasby, 1996). Prefrontal, parietal and cortical brain areas have been implicated to be involved in attentional processes with an increased activation found in these areas following tasks measuring attention (for example visual tracking) (Culham, Cavanagh, & Kanwisher, 2001; Sauseng et al., 2005).

2.6.2.2 Executive functions

Executive functions can be described as a group of capacities that enable an individual to successfully engage in independent, purposive and self-serving behaviour (Lezak, 2004). These functions are often referred to as ‘higher-order’ cognitive processes and include planning, organisation, strategy selection, set-shifting, problem-solving, inhibition, and performance monitoring (Fossati, Ergis, & Allilaire, 2002). Assessment of executive functions has
previously relied on tasks that have been shown to be impaired in individuals with frontal lobe damage, for example, the Wisconsin Card Sorting Test (Anderson, Damasio, Jones, & Tranel, 1991), the Tower of London Test (van den Heuvel et al., 2003), and verbal fluency tests such as Controlled Oral Word Association Test (Loonstra, Tarlow, & Sellers, 2001). However, more recently, it has been found that executive functions are not entirely dependent upon frontal lobe function and are regulated by neural networks involving different brain areas with co-ordinated complex functions to achieve a singular goal (Elliott, 2003).

2.6.2.3 Learning and memory

The main classifications of memory are working memory (also referred to as short-term memory) and long-term memory (Baddeley & Hitch, 1974). Working memory involves temporarily holding and manipulating limited amounts of information without thorough processing (in the current study tasks assessing working memory have been categorised under the domain ‘Executive Functions’). After initial acquisition of information, the next part of the memory process involves transferring encoded material to long-term memory, which is known as consolidation. Research suggests that the primary brain area responsible for memory consolidation is the hippocampus. Long-term memory is categorised into declarative memory (explicit memory) and procedural memory (implicit memory). Declarative memory consists of: i) semantic or factual memory, and ii) episodic memory or memory involving events and personal experiences (Lezak, 2004). Typically, learning and memory tests are further divided into verbal and visuospatial sub-categories. Some commonly used tasks to assess learning and memory are the Rey-Auditory Verbal Learning Test (M. Schmidt, 1996), the California Verbal Learning Test (Delis, Freeland, Kramer, & Kaplan, 1988), Verbal Paired Associates Test (Uttl, Graf, & Richter, 2002) and the Rey-Osterrieth Complex Figure Test (Shin, Park, Park, Seol, & Kwon, 2006). Learning and memory are complex processes that are coordinated by several
brain regions (e.g. the medial temporal lobe, frontal lobe, cerebellum, amygdala, neocortex and the striatum) (Lezak, 2004).

2.6.2.4 Psychomotor speed

Psychomotor speed is defined as the amount of time taken by an individual to detect and process a signal and to prepare and respond to a stimulus. Generally, tests assess reaction time (processing speed) and/or motor function, as motor function integrity affects speed on the cognitive task. Some examples include the Coin Rotation Test (Mendoza, Apostolos, Humphreys, B., & O'bryant, 2009), the Trail Making Test (Part A) (Bowie & Harvey, 2006) and the Digit Symbol Substitution Test (McLeod, Griffiths, Bigelow, & Yingling, 1982).

2.6.3 Key areas impaired in depression

Diminished concentration or indecisiveness is one of the main diagnostic items for MDD (DSM-5). In accordance with this finding, several systematic reviews examining cognitive deficits in depression have consistently found impairment in various cognitive domains, however, without consensus regarding impairment in specific domains (Austin, Mitchell, & Goodwin, 2001; Burt, Zembar, & Niederehe, 1995; Douglas et al., 2011; Marazziti, Consoli, Picchetti, Carlini, & Faravelli, 2010; Porter et al., 2015; Rock et al., 2013; H. Snyder, 2013; Thomas et al., 2009; Zalkanis et al., 1998).

Overall, studies have shown significant cognitive deficits in attention and executive functioning (including inhibition, problem-solving and planning) (Fossati, Ergis, et al., 2002; Marazziti et al., 2010), verbal and visuospatial learning and memory (Austin et al., 2001; Douglas, Porter, Knight, & Alsop, 2013; Golinkoff & Sweeney, 1989; Marazziti et al., 2010; Sarosi et al., 2008; Thomas et al., 2009), in individuals with depression compared with healthy control participants. Since different cognitive domains may overlap, it may be that memory disturbance is related to attention deficits or executive function impairment without a specific deficit limited to a single
domain (Marazziti et al., 2010). Some studies suggest that cognitive impairment in specific domains (including episodic memory, executive function and psychomotor speed) is positively associated with symptoms of mood disturbance (Fossati, Coyette, Ergis, & Allilaire, 2002; McDermott & Ebmeier, 2009), however, methodological limitations in these studies limit the ability to draw meaningful conclusions related to differences in cognitive domains, as summarised in a review by Porter et al. (2015).

In a study examining cognitive function in severe depression, widespread neuropsychological impairment was found in inpatients with depression compared with healthy control participants, with effect sizes ranging from moderate to large (Cohen’s $d$: 0.6 to 1.1) (Douglas et al., 2011). Studies in medication-free samples with mild-to-moderate depression have reported small to moderate effect size differences (most effect sizes were small-moderate ranging between 0.04-0.61) (Bourke et al., 2012; Porter et al., 2003). In addition, a recent study by Douglas et al. (2018) found the prevalence of cognitive impairment to be the highest in inpatient samples with current symptoms of severe depression compared with outpatients in mild-moderate depressive episodes. Cognitive impairment has been found to have a similar magnitude in patients with bipolar disorder in comparison with patients with MDD (Bora, Yucel, & Pantelis, 2010).

Inconsistent findings between cognitive studies of MDD in terms of the specific domains affected may be due to variability in study design, inclusion of depressed patients with different severity levels, lack of power, comorbid psychiatric disorders, depression rating scale selection and sensitivity of cognitive test batteries included (Castaneda, Tuulio-Henriksson, Marttunen, Suvisaari, & Lonnqvist, 2008). Additionally, cognitive function may be solely determined in terms of statistical significance, rather than a reflection of the actual difference in effect size. Studies have proposed that cognitive impairment is present in only a minority of patients with MDD while the remaining majority show normal cognitive functioning (Douglas et al., 2018; Grant, Thase, & Sweeney, 2001; Iverson, Brooks, Langenecker, & Young, 2011; Rohling,
Green, Allen, & Iverson, 2002), which has implications for interpretation of findings based on effect sizes or statistical significance (Burt et al., 1995; Iverson et al., 2011).

To summarise, although deficits in executive function, learning and memory, and psychomotor speed have been more commonly reported in patients with MDD, overall, there is little consensus regarding findings related to impairment in specific domains. Evidence suggests that broad moderate deficits rather than domain-specific deficits across a range of domains are found in individuals with MDD (Porter et al., 2015).

2.6.4 Cognitive impairment in depression: Longitudinal findings

Evidence suggests that cognitive impairment may persist after recovery from depression in many individuals. Whilst individuals in an acute episode of depression have been found to show worse performance in most cognitive domains, compared with healthy individuals, many studies including reviews have also demonstrated that depression may have a detrimental and residual impact on cognitive function (Bora, Harrison, Yucel, & Pantelis, 2013; Castaneda et al., 2008; Douglas & Porter, 2009; Hasselbalch et al., 2011; Jaeger et al., 2006; Paelecke-Habermann, Pohl, & Leplow, 2005; Reppermund, Ising, Lucae, & Zihl, 2009; Rock et al., 2013; Trivedi & Greer, 2014). However, an understanding of findings in reproductive-aged female populations is very limited, which is the focus of the current thesis. A review found that the risk for cognitive impairment is worsened with each depressive episode while the individual is in recovery (Porter et al., 2015), and that possible vascular and neurodegenerative factors may play a substantial role (Bora et al., 2013), however, more longitudinal prospective studies are required to ascertain this hypothesis (Hasselbalch et al., 2011). Rock et al. (2013) in a meta-analytical systematic review (including 24 studies for the review and six studies for the meta-analysis), found significant cognitive deficits in executive function and attention (including moderate effect sizes) and non-significant deficits (small effect sizes) in memory to persist in recovered patients with depression. The study, thus, concluded that impairment in at least in
some cognitive domains is a core feature of depression and is dissociable from symptoms of low mood in remission. Similarly, another study found no association between depression severity and any measures of cognitive function and suggested that depression and cognitive function are separable constructs (Reppermund et al., 2009). Thus, rather than being a secondary symptom of depression, cognitive function and mood may be independent features of the same disorder.

There is, however, some evidence that certain aspects of cognitive function may improve following treatment of mood symptoms in MDD (Constant et al., 2005; Douglas & Porter, 2009). Constant et al. (2005) found a beneficial effect of antidepressant treatment (Sertraline) over a period of seven weeks on psychomotor slowing associated with attentional bias and executive function in young individuals with MDD ($n = 20$) compared with healthy control participants ($n = 26$). Douglas et al. (2009), in a systematic review (including 30 studies), found improvement in mood to be associated with improvement in some cognitive domains and found age to play a crucial role. In younger adult populations, improvement in mood was significantly associated with better outcomes on measures of verbal fluency and verbal learning and memory, however, impairment in executive functions and attention were found to persist across treatment.

In summary, pronounced cognitive deficits are evident in individuals suffering from current depression and those in remission; with evidence suggesting that some aspects of cognitive impairment appear to improve following the treatment of symptoms of depression, while other domains may remain impaired. Much of the literature has examined this relationship in elderly individuals, and since the focus on the current thesis is on premenopausal females, literature related to cognitive impairment in depressed females of reproductive age will be discussed in the following sections and chapters.
2.7 DEPRESSION AND EMOTION PROCESSING

The terms ‘emotions’ and ‘moods’ are often used interchangeably, but differ in important ways. Moods are longer-lasting and can activate specific emotions (Ekman, 2007), therefore, it may be hypothesised that a low mood found in MDD may affect an individual’s experience and perception of emotions. The ability to detect and decode others’ facial emotional expressions is vital for social functioning and for promoting interpersonal relationships. Facial expressions communicate an individual’s mental state and related emotional cues; and may influence behavioural responses and regulate mood (M. Phillips, Drevets, Rauch, & Lane, 2003). Facial emotion processing involves empathy, which refers to sensitivity to, and understanding of, the mental states of others (Bourke, Douglas, & Porter, 2010; Leppänen, 2006; Stuhrmann, Suslow, & Dannlowski, 2011; Tarnowski, Kołodziej, Majkowski, & Rak, 2017). Similar to findings related to cognitive function in depression, short-term and long-term abnormalities (in recovered individuals) in emotion processing have also been found in individuals with MDD and in individuals with an increased risk of developing MDD (Leppänen, 2006; Leppänen, Milders, Bell, Terriere, & Hietanen, 2004; Surguladze et al., 2004) reviewed by (Bourke et al., 2010). Specific findings related to facial emotion processing in MDD will be discussed in the following sections, followed by a discussion of neuroimaging findings related to cognitive impairment and impairment in emotion processing found in MDD.

2.7.1 Measures of facial emotion processing

A standardised set of stimuli including six universally-recognised emotional expressions forms the basis of measures most widely used to assess facial emotion processing (Ekman & Friesen, 1971). Two main categories of tasks are used to assess facial emotion processing, namely, identification (emotional labelling) and discrimination (categorising) paradigms (Bourke et al., 2010). Tasks often involve morphed face paradigms using computerised combinations of varying emotional content (from 0 to 100% in increments of 10%) presented in a random order.
(Young et al., 1997). Accuracy, response speed and type of misclassification of ambiguous or neutral expressions are principal outcomes of facial emotion processing paradigms.

### 2.7.2 Facial emotion recognition accuracy in depression

Evidence from studies (Douglas & Porter, 2010; Gotlib, Krasnoperova, Yue, & Joormann, 2004; Leppänen et al., 2004; Suslow, Dannlowski, Lalee-Mentzel, Donges, & Arolt, 2004) including reviews (Bediou, Saoud, Harmer, & Krolak-Salmon, 2009; Bourke et al., 2010; Leppänen, 2006; Stuhrmann et al., 2011) suggests that depression involves reduced accuracy and reduced reaction time in recognising emotions including sadness, happiness and disgust, however, many other studies have not found these effects.

Accuracy in recognising some facial expressions of emotion has been found to be related to treatment outcome and recurrence of depressive illness in some studies (Douglas & Porter, 2009; Harmer, Bhagwagar, et al., 2003; Joormann & Gotlib, 2006; LeMoult, Joormann, Sherdell, Wright, & Gotlib, 2009; Stuhrmann et al., 2011).

Treatment response to antidepressant medication has been shown to significantly improve aspects of facial expression recognition. In 68 patients with severe depression, recognition of angry facial expressions significantly improved in antidepressant treatment responders compared with non-responders (Douglas et al., 2011). Mikhailova et al. (1996) found recognition accuracy of happy, sad and neutral faces to improve following antidepressant treatment in male patients with depression, although this study did not include a comparison group. Tranter et al. (2009) found significant increases in recognition accuracy of emotional expressions including disgust, happiness and surprise following two weeks of treatment (Citalopram and Reboxetine) in 59 patients with depression, which was not subject to further change at six weeks. Additionally, a significant association was observed between an increased accuracy in recognition of happy faces (at 2 weeks) and improvement in clinical state following
6 weeks of treatment in this study. A further study found impairment in recognition of happy facial expressions (measured by standard facial stimuli with varying intensities of happiness) in untreated patients with MDD ($n = 19$) compared with a healthy control group ($n = 19$); which was found to reverse following treatment (Fluoxetine) (Fu et al., 2007). Overall, these findings suggest that antidepressant treatment may benefit facial emotion recognition accuracy independent of the affective state of stimuli.

### 2.7.3 Neutral misinterpretation bias in depression

Misclassification of ambiguous or neutral facial expressions has been found in MDD (Bourke et al., 2010; Douglas & Porter, 2010). Individuals with MDD tend to misclassify neutral or ambiguous faces as negative or sad, and misinterpret happy faces as neutral (Elliott, Zahn, Deakin, & Anderson, 2010; Harmer, O’Sullivan, et al., 2009; Stuhrmann et al., 2011). These findings fit with the psychological theories of depression including negative cognitive biases (Beck, 1967). Cognitive theories posit that individuals with depression are likely to not only misinterpret ambiguous events as negative but also to show an enhanced memory for negative events, together with an ability to intensify negative material in memory tasks by filtering out of positive stimuli (Beck, 1979; Bouhuys, Geerts, & Gordijn, 1999; Elliott et al., 2010; Gotlib, Kasch, et al., 2004; Gotlib, Krasnoperova, et al., 2004; Leppänen et al., 2004; Surguladze et al., 2004).

In contrast to the cognitive theories of depression, neurobiological theories relate symptoms of depression to a chemical imbalance in neurotransmitters, therefore including pharmacotherapy as first-line treatment. Some evidence suggests that antidepressant treatment has immediate effects in the brain and improves a negative interpretation bias in individuals with MDD much earlier than its effects on mood (Harmer, O’Sullivan, et al., 2009). Studies have shown that a single administration of antidepressant medication increases processing of positively valenced material in healthy individuals (Harmer, Hill, Taylor, Cowen, & Goodwin, 2003) and improves
a negative interpretation bias in healthy individuals without MDD (Norbury et al., 2009), suggesting that antidepressants may reverse impairment in emotion processing found in MDD. Douglas et al. (2011) found a significant reduction in negative interpretation bias (following two weeks of treatment) in 68 individuals with MDD. Following two weeks of antidepressant treatment, non-responders were significantly less likely to misinterpret neutral faces as negative (sad) compared with treatment responders but not compared with healthy control participants.

2.7.4 Attentional biases

An attentional bias towards positive emotional faces (for example, happy facial expressions) or away from negative emotional faces (for example, sad facial expressions) has been reported in MDD (Bourke et al., 2010; Leppänen, 2006). Such an attentional bias may be one of the important factors involved in abnormal emotion processing which may adversely affect interpersonal relationships in the life of an individual with MDD. Joorman et al. (2007) in a dot-probe paradigm, found a selective avoidance of sad faces in currently and previously depressed individuals and found increased attention toward happy faces in healthy controls, which was not observed in individuals with MDD. On the other hand, in another study involving a dot-probe paradigm (Gotlib, Krasnoperova, et al., 2004), female patients with MDD showed an attentional bias towards sad facial expressions compared with happy, fearful and angry facial expressions. In another paradigm involving scanning several faces and discerning differences between them (Face-in-the-Crowd Task), Suslow et al. (2001) found no difference between individuals with depression ($n = 15$) and healthy controls ($n = 15$) on accuracy of detecting sad faces, however, individuals with depression were found to have a significantly slower response time to positive faces, suggesting a reduced attention to positive stimuli in depression. Overall, the existing literature suggests an attentional bias away from positive and toward negative facial emotional stimuli in individuals with MDD (Bourke et al., 2010). However, attentional bias studies have many different outcome variables (for example, reaction time, accuracy and bias).
and measure different emotional expressions, therefore, generalised conclusions beyond those made by individual papers are difficult to draw.

### 2.7.5 Neuroimaging findings related to impairment in cognitive function and emotion processing in depression

Neuroimaging studies have reported smaller hippocampal volumes in individuals with depression (Bremner et al., 2000), which has been related to impairment in memory also found in depression (van der Flier et al., 2004). Increased and sustained amygdala activity (Drevets, Bogers, & Raichle, 2002; Drevets et al., 1992; J. Hamilton & Gotlib, 2008; Siegle, Steinhauser, Thase, Stenger, & Carter, 2002) and volumetric amygdala abnormalities (increased volume in medicated depressed patients and decreased volume in unmedicated patients) have been found in individuals with depression (J. Hamilton, Siemer, & Gotlib, 2008). Dorsal prefrontal cortex and anterior cingulate abnormalities are reported in individuals with depression along with impaired cognitive function (Haldane & Frangou, 2014; Liotti & Mayberg, 2001), however, further studies are required to fully determine the functional changes associated with cognitive impairment in major depression.

Structural and frontal abnormalities including abnormalities in key brain regions including increased activity in the amygdala, orbitofrontal cortex and the striatum (responsible for emotional identification and production) and decreased activity within the dorsolateral prefrontal cortex and the anterior cingulate cortex (involved in emotion regulation) have been associated with impairment in emotion processing (Haxby, Hoffman, & Gobbini, 2002; Stuhrmann et al., 2011). Studies have also implicated these brain regions in the aetiology of MDD (M. Phillips et al., 2003; M. Phillips, Ladouceur, & Drevets, 2008). Additionally, abnormalities in brain regions such as the hippocampus, prefrontal cortex, and the somatosensory cortex have been linked with impairment in emotion processing (Haxby et al., 2002). Specific impairments in emotion processing such as mood-congruent processing bias
(hyperactivation toward negative facial stimuli and hypoactivation toward positive facial stimuli) have been related to abnormalities in the amygdala, ventral-striatum, anterior cingulate cortex, parahippocampal gyrus, insula, fusiform face area and putamen in individuals with major depression compared with healthy controls (Fusar-Poli et al., 2009; Leppänen, 2006; Stuhrmann et al., 2011). However, there are inconsistencies in findings related to prefrontal brain areas which have been speculated to be due to varying paradigms and heterogeneous patient samples (Stuhrmann et al., 2011).

It has been suggested that antidepressant medication may modulate the neural networking involved in emotion processing (including an increased amygdala activity) which may help normalise a negative bias found in mood and anxiety disorders. Antidepressant treatment was found to significantly reduce amygdala activity in response to emotional faces, particularly related to negative emotional faces (Harmer, Mackay, Reid, Cowen, & Goodwin, 2006; Norbury et al., 2009; Sheline et al., 2001) and increase dorsolateral prefrontal cortex activity in response to unconsciously shown fearful faces (Fales et al., 2009) in patients with depression compared with healthy controls.

Overall, neuroimaging evidence in facial emotion processing studies suggests that the abnormal neural activation may be responsible for impairment in emotion processing observed in patients with MDD, and that neural abnormalities may be reversed with antidepressant treatment, which may help normalise the negative bias found in MDD.

2.8 ANXIETY – COMORBIDITY AND PREVALENCE OF ANXIETY IN DEPRESSED SAMPLES

Anxiety symptoms are commonly observed in patients with depression, with more than 70% of individuals with MDD also diagnosed with a sub-type of anxiety disorder (Gorman, 1996;
Hirschfeld, 2001; Kessler, Merikangas, & Wang, 2007; Lamers et al., 2011; Wolitzky-Taylor et al., 2014). The US National Comorbidity Survey reported that 59.2% of individuals with MDD also experienced at least one type of an anxiety disorder (Kessler et al., 2003). These results were broadly consistent with the New Zealand Mental Health Survey (Te Rau Hinengaro) which reported that the most common mental health comorbidities occurred between symptoms of anxiety and depression, with approximately 49.6% of those individuals suffering from MDD also experiencing some symptoms of anxiety (Oakley-Browne et al., 2006). Risk factors for both psychiatric conditions may include genetic and environmental factors or an interaction between the two, impacting on clinical and treatment outcomes (Pollack, 2005).

Limited research has examined the impact of comorbid anxiety and depression on cognitive impairment, and with inconclusive results (Herrera-Guzmán et al., 2009; Nitschke & Heller, 2005). Cognitive data from extant research indicates that anxiety is not a homogenous entity attempts to examine its neural circuitry should take into account comorbidity with other disorders (Nitschke & Heller, 2005). Some studies have found individuals with depression and comorbid anxiety to experience specific cognitive impairment. In a study examining the effect of comorbid anxiety on cognitive function in individuals with depression, it was found that memory was impaired, regardless of whether or not comorbid anxiety was present. However, specific deficits in executive function and psychomotor speed were found only in the depressed group with comorbid anxiety, compared with the depression only group and the healthy control group (Basso et al., 2007). In another study, cognitive impairment was found to be common in young adults with MDD and comorbid anxiety (Castaneda et al., 2008). The profile of cognitive impairment was found to be dependent on the type of anxiety. For example, executive function impairment was common in individuals with MDD only, however, in those individuals with MDD and comorbid obsessive-compulsive disorder, deficits in executive function and visual memory were found. With regard to changes in cognitive impairment following treatment of
depression with comorbid anxiety, one study found that individuals with MDD and comorbid anxiety showed less improvement in cognitive function than individuals with MDD only, following antidepressant treatment (Herrera-Guzmán et al., 2009). Thus, overall findings suggest that the occurrence of both anxiety and depression together may be associated with worse cognitive impairment compared with individuals with either diagnosis alone.

2.9 TREATMENT STRATEGIES FOR DEPRESSION

2.9.1 Pharmacological treatment

Historically, depression was thought to be a disease of the brain or the mind, and hence, treatment was carried out in accordance with this aetiological understanding, involving either pharmacological intervention or psychological therapy. The former consists of medications that alter key neurotransmitter levels (for example, serotonin noradrenalin or dopamine) in patients (see Box 2.1), however, while antidepressants can treat the symptoms of depression they may not always address its causes. Therefore, antidepressant medication is often used simultaneously with psychotherapy to treat more severe depression. However, depressive disorders are challenging to treat. Current first-line treatments for MDD have limited efficacy (Arroll et al., 2009; Penn & Tracy, 2012; Sugarman, Loree, Baltes, Grekin, & Kirsch, 2014), with up to 40% of depressed patients failing to demonstrate a response to first-line antidepressant drug treatment, and many of those who do respond ultimately relapse (Arroll et al., 2009; Gaynes, 2009; Khan & Brown, 2015; Nemeroff, 2007). The effect size of current antidepressant treatment trials of patients with MDD is approximately 0.30 which is modest (Gibertini, Nations, & Whitaker, 2012; Khan & Brown, 2015). Furthermore, the compliance rate with antidepressants can be low, with up to 50% of patients discontinuing antidepressant treatment prematurely (Sansone & Sansone, 2012; Trivedi, Lin, & Katon, 2007). Additionally, antidepressants have unwanted side-effects (Arroll et al., 2009; Eyding et al., 2010), and do
little to improve cognitive impairment (Airaksinen et al., 2006; Porter et al., 2016; Shilyansky et al., 2016). The following section (see Box 2.1) will describe the main treatment agents involved in anti-depressant treatment for MDD.

**Box 2.1**

**Different Pharmacological Strategies for Major Depressive Disorder**

<table>
<thead>
<tr>
<th>Pharmacological treatment of depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>All antidepressant medication agents increase serotonin, norepinephrine and/or dopamine levels via different mechanisms. First-generation antidepressants comprise of tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs). The subsequent group of medication became known as second-generation agents, and are considered more sophisticated in terms of mode of action and safety (Amick et al., 2015; Williams et al., 2000). These include selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenaline reuptake inhibitors (SNRIs) and the noradrenaline-dopamine reuptake inhibitors (NDRIs) (Koenig &amp; Thase, 2009).</td>
</tr>
</tbody>
</table>

**Tricyclic antidepressants:** Tricyclic antidepressants block the reuptake of serotonin and noradrenaline transporters and the most common agents in this category include Clomipramine and Imipramine. Some side-effects include dry mouth, weight gain, and sexual dysfunction.

**Monoamine oxidase inhibitors:** Monoamine oxidase inhibitors block the actions of monoamine oxidase enzymes that are responsible for the breaking down of neurotransmitters including serotonin, dopamine and noradrenaline. Decreased levels of these neurotransmitters are associated with MDD. Therefore, by inhibiting the effects of monoamine oxidase enzymes, MAOIs increase neurotransmitter levels which help improve
mood. Medications within this class include Phenelzine, Tranylcypromine and Isocarboxazid.

**Selective serotonin reuptake inhibitors** – Selective serotonin reuptake inhibitors are considered first-line antidepressant treatment in New Zealand and include Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine and Sertraline. These medications selectively inhibit the reuptake of serotonin. Unlike TCAs, SSRIs can be started at a therapeutic dose and cause fewer side effects such as constipation, gastrointestinal issues, dry mouth and blurry vision (Koenig & Thase, 2009). However, other side effects include agitation or motor restlessness, and increased suicidality.

**Serotonin-noradrenaline reuptake inhibitors**: These medications increase both serotonin and noradrenaline levels and include Venlafaxine, Desvenlafaxine and Duloxetine. Common side-effects include nausea, dizziness, headache and insomnia.

**Noradrenaline and dopamine reuptake inhibitors**: These medications block noradrenaline and dopamine reuptake transporters, respectively. Bupropion is the most commonly used NDRI and combination therapy of Bupropion with other second-generation antidepressants has been shown to improve outcomes in patients for whom antidepressant monotherapy has failed to work (Moreira, 2011). There is an associated risk of epileptic seizures with the use of NDRIs (Penn & Tracy, 2012) and other side effects include anxiety, insomnia, loss of appetite and weight loss.

### 2.9.2 Psychotherapy

A recent update of current care guidelines for management of depression and treatment research suggests that combining antidepressant medication with psychotherapy is more effective that sole treatment with either (Cuijpers et al., 2013; Harmer, Goodwin, & Cowen, 2009; Isometsä
et al., 2015), however, other studies have not found similar results (Kocsis, Gelenberg, Rothbaum, & et al., 2009; von Wolff, Hölzel, Westphal, Härter, & Kriston, 2012). Antidepressants were deemed suitable for severe depressive symptoms, however, brief psychotherapies including cognitive, interpersonal, or problem-solving were thought to be more beneficial in mild-to-moderate depression. Cognitive Behavioural Therapy (CBT) has been found to be generally efficacious for the treatment of depression (Cuijpers et al., 2013) and aims to identify and modify dysfunctional thinking or behavioural patterns; substituting them with more functional and accurate thoughts and behaviours (Beck, 1979). Interpersonal therapy (IPT) helps individuals understand problematic interpersonal issues and learn healthy ways of expressing emotions and communicating needs (de Mello, de Jesus Mari, Bacaltchuk, Verdeli, & Neugebauer, 2005). The Australia and New Zealand Clinical Practice Guidelines (2004) for the treatment of depression report that all recognised antidepressant treatment should precede psychological treatment in severe cases of depression, however, in moderate-severe depression, CBT and IPT are equally effective as medication.

2.10 KEY POINTS

Major depressive disorder is a serious and pervasive mood disorder affecting twice as many females as males in adulthood. In at least some females, depression may have hormonal underpinnings. Cognitive impairment and deficits in facial emotion processing are well-established findings in MDD. Whilst antidepressant treatment may offer short-term and long-term benefits, it still has limited benefits for individuals with mild-moderate depression and does not always help improve cognitive deficits. It is, therefore, important to develop comprehensive treatment strategies that optimise overall function, particularly in patients whose symptoms of depression may be influenced by hormonal abnormalities, such as in females with PCOS.
CHAPTER 3

ANDROGENS IN FEMALES

3.1 INTRODUCTION

Since the word ‘androgen’ has its origins in the Greek word ‘andros’ meaning man, this group of sex hormones is often mistakenly believed to exist only in males. However, androgens are important sex steroids in females as well as males. This class of hormones has important implications, mainly in sexual development, for maintaining sexual desire and in their conversion (via aromatisation) into oestrogen. Females secrete greater amounts of androgens than oestrogens (Burger, 2002).

While the role of oestrogens (female sex hormones) in the female brain has received a great deal of research attention, knowledge of the effect of testosterone and other androgens on the female brain remains more limited. Increasing evidence suggests a role of androgens in mood and cognitive function (Beauchet, 2006; Cherrier, 2005; Davison & Davis, 2003; Gooren, 2007; Janowsky, 2006; Zitzmann, 2006). However, few studies have examined this relationship in reproductive-aged females. It has been established that reproductive-aged females have the greatest risk for developing depressive disorders compared with non-reproductive-aged females and males (Noble, 2005). Therefore, it is imperative to understand the main functions and mechanisms of androgens in reproductive-aged females to further current understanding of mood and associated cognitive impairment in females.

For clarity in this thesis, sex hormones (also known as sex steroids, gonadocorticoids or gonadal steroids) refer to both testosterone and oestrogen. The main types of androgens, production
rates and functions, and effects of androgen-excess related disorders in females will be discussed in the following sections.

3.2 NORMAL ANDROGEN FUNCTION IN HEALTHY FEMALES

Women produce approximately 66% of the total androgens found in men (Burger, 2002). This group of hormones is synthesised in the endocrine tissues from cholesterol or via aromatisation from other androgens or androgen precursors, peripherally, including the liver, gonads and adrenal cortex in females (Hodgson & Braunstein, 2006). Androgens are vital for maintaining muscles and bones, maintaining positive protein balance, regulation of the reproductive tract and kidney function, and in stimulating sexual development (important during puberty for sebum production and hair growth) and sexual desire (Swerdloff, Wang, Hines, & Gorski, 1992). An important function of androgens is that they are precursors to oestrogen, responsible for oestrogen synthesis (via conversion of testosterone or DHEA to oestrogen) (Burger, 2002). Major biological events in women including adrenarche, menarche, sexuality, fertility and menopause are mediated by androgens (Hodgson & Braunstein, 2006). Androgens also play a role in the organisational and activational aspects of brain function.

3.3 TYPES AND SOURCES OF ANDROGENS

The main androgens in women, in decreasing order of serum concentration, are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione (A-dione), androstenediol (A-diol), testosterone (Total and Free), and dihydrotestosterone (DHT) (Burger, 2002; Davison & Davis, 2003). Testosterone and DHT are the only androgens that bind to the androgen receptor, whereas DHEA, DHEAS and A-dione are considered as pro-androgens and require conversion to testosterone in order to have an androgenic effect on the
body (Burger, 2002). Androgens are produced in the adrenal glands (in the zona reticularis of the adrenal cortex) (25%), ovaries (in the ovarian stroma) (25%), fat cells and through peripheral conversion of precursor androgens (DHEA and A-dione) (50%) in females (Abraham, 1974; Davison, Bell, Donath, Montalto, & Davis, 2005; Endoh, Kristiansen, Casson, Buster, & Hornsby, 1996; Hamson, Roes, & Galea, 2016; Haning et al., 1991; O'Neill, 2012). Androgen precursors such as DHEA, DHEAS, A-dione, A-diol and 11 β-hydroxyandrostenedione (11OHA) (also known as C-19 steroids) have their sources mainly in the adrenal glands (Rege et al., 2013), whereas the main types of androgens including testosterone (Total, Free and DHT) are primarily produced in the ovaries. The next section will discuss the different types of androgens and pro-androgens.

**Box 3.1**

**Forms of Testosterone**

<table>
<thead>
<tr>
<th><strong>Total Testosterone</strong></th>
<th>98 percent of testosterone is bound to Sex Hormone Binding Globulin (SHBG; See Section 3.3.4) and albumin in women and is referred to as Total Testosterone.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Free Testosterone</strong></td>
<td>The lesser fraction of testosterone (1 to 2 percent) is estimated to be biologically available or in an active form and is known as Free Testosterone. This measure is also referred to as Bioactive or Bioavailable Testosterone.</td>
</tr>
<tr>
<td><strong>Free Androgen Index</strong></td>
<td>A ratio of Total Testosterone to SHBG is known as Free Androgen Index (FAI) and is considered a reliable way of calculating bioavailable testosterone in women (Total T/SHBG x 100= FAI).</td>
</tr>
<tr>
<td><strong>Dihydrotestosterone</strong></td>
<td>Dihydrotestosterone (DHT) is primarily a peripheral product of testosterone conversion and circulates in low concentrations in serum. DHT is non-aromatisable and therefore cannot be converted to oestrogen.</td>
</tr>
</tbody>
</table>

At present, there is no consensus regarding a single, reliable measurement standard of androgen levels (Rosner, Auchus, Azziz, Sluss, & Raff, 2007). Direct assays of testosterone are
considered unreliable and expensive, therefore assays are generally established after extraction and chromatography, or alternatively are derived through equations estimating Free or Total Testosterone levels (Al Kindi, Al Essry, Al Essry, & Mula-Abed, 2012; K. Miller et al., 2004; Sodergard, Backstrom, Shanbhag, & Carstensen, 1982; Vermeulen, Verdonck, & Kaufman, 1999).

3.3.1 Testosterone

The most potent androgen, testosterone, is produced by the ovarian stroma (25%) and the adrenal cortex (25%) in women, while the remaining production results from peripheral conversion from several precursors including circulating A-dione, DHEA and DHEAS (Baird, Horton, Longcope, & Tait, 1968; Burger, 2002; Davison & Davis, 2003; Longcope, 1986). It is estimated that 98% of Total Testosterone is bound to SHBG and a lesser fraction, known as Free Testosterone, is biologically available and unbound. Total Testosterone levels are known to be weakly correlated with Free Testosterone levels. The metabolically active portion, Free Testosterone (also known as bioavailable or bioactive testosterone), provides a more predictive assay of androgenic status, particularly in women with endocrine disorders involving androgen excess (Huang, Brennan, & Azziz, 2010; Vermeulen et al., 1999).

Free Androgen Index (see Box 3.1) is considered to be a reliable measure of Free Testosterone in women. Lower levels of SHBG are associated with higher FAI levels (Burger, Dudley, Cui, Dennerstein, & Hopper, 2000). Therefore, any factors affecting SHBG production also influence testosterone levels. These may include factors such as obesity, hyperinsulinemia, glucocorticoids, oral oestrogen, thyroxine, and menopause, which decrease SHBG levels, and in turn, may lead to elevated Free Testosterone levels (Burger et al., 2000; Davison & Davis, 2003; Simó, Sáez-López, Barbosa-Desongles, Hernández, & Selva, 2015; Thijssen, 1988).
Testosterone has important physiological consequences either directly or via aromatisation (conversion via enzyme aromatase) to oestradiol in women (Davis & Wahlin-Jacobsen, 2015). Two major metabolites of testosterone that show significant physiological activity are DHT and oestrogen, therefore, testosterone can exert its effects through the androgen receptor or the oestrogen receptor.

Testosterone can be converted to oestradiol in the brain by the aromatase enzyme, found in the hippocampus and amygdala (Janowsky, 2006) or in adipose tissue (Kim & Halter, 2014). DHT, another metabolite of testosterone (via the action of 5α-reductase), is considered to be a more potent androgen compared with testosterone, since it binds with greater affinity to androgen receptors (Hamson et al., 2016). It is a peripheral product of testosterone conversion and is found in low circulating serum concentrations (Abraham, 1974). However, unlike testosterone, DHT is non-aromatisable (does not convert to oestrogen). In women, testosterone varies with menstrual phases, with lowest concentrations seen early in the follicular phase of the menstrual cycle, then rising to a mid-cycle peak before lowering in the luteal phase (but still remaining higher than early follicular phase) (Abraham, 1974).

### 3.3.2 Dehydroepiandrosterone and Dehydroepiandrosterone sulphate

Both DHEA and DHEAS are abundant endogenous adrenal androgens produced by the adrenal cortex which contributes to 80% of DHEA production and more than 90% of DHEAS synthesis (Abraham, 1974; Geese & Blanchard Raftogianis, 2001; Luu-The, Dufort, Paquet, Reimnitz, & Labrie, 1995; Spark, 2002). DHEA is mainly secreted by the adrenal zona reticularis (50%), the ovarian theca (20%) and is also synthesised from circulating DHEAS (30%) (Labrie, Martel, & Balser, 2011; Longcope, 1986; Luu-The et al., 1995). Production is stimulated by ACTH, which is secreted by the pituitary gland (Ogino, Miyagawa, & Iguchi, 2016). Evidence suggests that DHEA is associated with age-related changes in immune function, mood and cognitive
function (Barrett-Connor, Mühlen, Laughlin, & Kripke, 1999; Herbert, 2007; Wolkowitz et al., 1999).

The sulphated form of DHEA, DHEAS, is mainly secreted by the adrenal glands (zona reticularis) and to a small extent by the ovaries. DHEAS is known to be a biologically weak androgen (Endoh et al., 1996), however, is a crucial source of peripheral androgen production (Burger, 2002). Its secretion is regulated by ACTH and may be influenced by prolactin, Insulin Growth Factor-1 (IGF-1) and oestrogen. DHEA/testosterone and DHEAS/testosterone ratios, however, have been shown to be age-invariant (Zumoff, Strain, Miller, & Rosner, 1995).

3.3.3 Androstenedione and Androstenediol

Androstenedione is secreted by the adrenal glands (50%) and the ovarian stroma (50%) and is a precursor to testosterone (Horton & Tait, 1966). It is a metabolite of DHEA and can also be produced intracellularly from DHEAS via DHEA (Burger, 2002). Androstenediol is a major metabolite of the potent androgen DHEA, secreted by the adrenal glands. It is also an androgen precursor and is capable of activating both androgen and oestrogen target genes (Miyamoto, Yeh, Lardy, Messing, & Chang, 1998).

3.3.4 Sex Hormone Binding Globulin

Sex Hormone Binding Globulin is a circulating steroid-transporting protein, produced by the liver, and binds tightly to androgens (mostly testosterone) and oestrogen (O'Neill, 2012; Simó et al., 2015). It binds reversibly and with great affinity to testosterone and DHT, and to a certain extent, with oestrogen. In its bound state, SHBG transports these hormones in the blood as biologically inactive forms (C. Li, Ford, Li, Giles, & Liu, 2010). The main fraction of testosterone, Total Testosterone, is bound to SHBG, unlike the small fractions of biologically active sex hormones in plasma (Free Testosterone). Androgenicity (physiological effect of androgens), therefore, depends mainly on Free Testosterone due to the high affinity of SHBG to
Total Testosterone. In females, SHBG concentrations are twice as high as males, indirectly proportional to testosterone levels, which, in turn, are ten-fold lower in females compared with males. SHBG controls the amount of testosterone that the body tissues can use, and therefore, any abnormalities in SHBG levels indicate testosterone abnormalities in women.

**Table 3.1**

*Androgen Production in Women*

<table>
<thead>
<tr>
<th>Type of androgen</th>
<th>Normal levels of androgens in women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Testosterone</td>
<td>0.1-1.2 ng/ml (0.3-2.7 nmol/L) 1 ng/ml = 3.47 nmol/L</td>
</tr>
<tr>
<td>Free Testosterone</td>
<td>0.6-6.8 pg/ml (&lt;50 pmol/L) 1 pg/ml = 3.47 pmol/L</td>
</tr>
<tr>
<td>FAI</td>
<td>&lt;80</td>
</tr>
<tr>
<td>DHEA</td>
<td>0.5-12.0 umol/µmol 3-35 nmol</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Premenopausal 0.5-12.0 umol/µmol 0.8-3.9 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Pregnancy 0.2-1.2 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Post-menopausal 0.1-0.6 µg/ml</td>
</tr>
<tr>
<td>DHT</td>
<td>Premenopausal 50-600 pmol/L 24-368 pg/ml</td>
</tr>
<tr>
<td>A-dione</td>
<td>Pre-menopausal (circulating concentrations) &lt;6 nmol/L 0.5-2 ng/ml (2-8 nmol)</td>
</tr>
<tr>
<td>SHBG</td>
<td>20-90 nmol/L 18-114</td>
</tr>
</tbody>
</table>

Source-(Canterbury Health laboratories), FAI- Free Androgen Index, DHEA-Dehydroepiandrosterone, DHEAS-Dehydroepiandrosterone sulfate, DHT- Dihydrotestosterone, A-dione- Androstenedione, SHBG- Sex Hormone Binding Globulin.
3.3.5 **Sex hormones and the blood brain barrier**

The blood brain barrier (BBB) separates the central nervous system from peripheral tissues and is a protective barrier for neurons in the brain (Banks, 2012). Integrity of the BBB is crucial for protecting the brain from toxic substances entering through the blood stream, and is ensured by multicellular systems including endothelial cells, astrocytes, pericytes among other systems (Almutairi, Gong, Xu, Chang, & Shi, 2016; Hampl, Bičíková, & Sosvorová, 2015). However, the BBB shows selective permeability toward sex hormones (Pardridge & Mietus, 1979). As sex hormones are small in size and are lipid-soluble, they are able to cross the BBB in both directions (Banks, 2012; Cornford, Braun, Oldendorf, & Hill, 1982; Pardridge, 1981). Current understanding is that concentration of androgens in the brain is limited to Free Testosterone but not Total Testosterone, as it is only Free Testosterone that is able to cross the BBB (Carruthers, 2009; Pardridge, 1986).

![Diagram](figure3_1.png)

**Figure 3.1- Steroidogenesis in the ovaries, adrenal glands and peripheral tissues of androgens in females.**

- **DHEAS**-Dehydroepiandrosterone sulfate, **DHEA**-Dehydroepiandrosterone, **A**-Androstenedione, **T**- Testosterone, **E2**- Estradiol, **DHT**- Dihydrotestosterone
3.3.6 Other Important Hormones

Hormones apart from androgens in reproductive-aged healthy females will be discussed in the following section (see Box 3.2).

Box 3.2
Other Important Hormones

**Oestrogen**

Oestrogens are primary female sex hormones with widespread biological actions. This group of hormones consists of chemically similar hormones including oestrone, oestradiol and oestriol. Oestrogens play an essential role in the development of female sexual characteristics and in the regulation of menstrual cycle and the reproductive system. Oestrogenic hormones regulate the menstrual cycle by controlling the growth of uterine lining during the first part of the cycle.

The main sources of production are the ovaries, adrenal glands and the fat tissues in females. Oestrogens can also be synthesised in non-reproductive tissues such as liver, heart, muscle, bone and the brain (R. Li, Cui, & Shen, 2014). The peripheral conversion of testosterone to oestrogen (via the enzyme aromatase) contributes to a small amount of total oestradiol, which is the most potent and prevalent oestrogenic hormone particularly during reproductive years (Arevalo, Azcoitia, & Garcia-Segura, 2015). Of the total oestradiol, 50% is protein bound and 3% is free (Kim & Halter, 2014).

It has been suggested by both animal and human studies that oestradiol and progesterone are both highly lipophilic and easily pass through the BBB (see Section 3.3.5) and regulate BBB permeability (Alkayed et al., 2001; Maggioli et al., 2016; Naderi, Khaksari, Abbasi, &
Maghool, 2015). Oestradiol and progesterone receptors are vastly expressed in brain areas related to cognitive function and emotion processing (Brinton et al., 2008; Gruber, Tschugguel, Schneeberger, & Huber, 2002; McEwen, 2002), however, a recent review found no consistent evidence related to the relationship between menstrual phases involving high or low oestrogen levels and sexually dimorphic cognitive skills in females (Poromaa & Gingnell, 2014).

**Progesterone**

Progesterone is a female sex hormone vital for supporting pregnancy by maintaining the uterine lining to receive a fertilised egg in the uterus. This hormone is mainly secreted by the ovaries, and in smaller quantities by the adrenal glands and the placenta (which continues to produce large quantities of progesterone throughout pregnancy). Levels are generally low before ovulation and rise after the egg is released from the ovary. If pregnancy occurs, progesterone levels remain elevated, otherwise a drop in levels is seen prior to menstruation.

Birth control pills or oral contraceptive pills use a progestin, a synthetic form of progesterone, which is structurally different to progesterone and works by preventing ovulation, thickening the mucus at the cervix to prevent sperm from entering and changing the womb lining to prevent pregnancy. Selman et al. (1997) reported that the administration of progestins in animal subjects resulted in suppression of the HPA axis. Progestins have been shown to act on the hypothalamus and the pituitary gland to prevent ovulation in healthy premenopausal females (Richter, Robinson, & Evans, 2002). Combination pills make use of both oestrogen and progestins for the same purpose (Duijkers et al., 2015).

**Anti-Mullerian Hormone**

Anti-Mullerian Hormone (AMH) is considered to be a quantitative marker for ovarian reserve indicating follicle depletion rates and ovarian ageing (Fiçicioğlu, Kutlu, Baglam, & Bakacak, 2006; Gruijters, Visser, Durlinger, & Themmen, 2003; Kevenaar et al., 2006;
Smeenk et al., 2007; van Rooij et al., 2004), independent of the menstrual cycle (La Marca et al., 2006). It is crucial for sex differentiation during foetal development and postnatally, it is expressed in the ovaries in females. Anti-Mullerian Hormone levels are generally lowest at birth and increase in a stable manner until adulthood in females and decrease subsequently, marking reproductive decline (M. Lee et al., 1996).

Studies suggest that AMH levels may be a more accurate marker of female reproductive status/fertility (particularly early menopause) compared with chronological age alone (van Disseldorp et al., 2008). Serum AMH levels have been found to be significantly higher in women with ovarian dysfunction (in women with endocrine disorders) (Dewailly et al., 2010; Pellatt et al., 2007) indicating increased follicle count and poor ovarian reserve (Cook, Siow, Brenner, & Fallat, 2002; Fanchin et al., 2003; Laven et al., 2004). Some studies have associated AMH with reduced oestrogen levels (Josso, di Clemente, & Gouédard, 2001). A recent study found elevated prenatal AMH levels to reprogram the foetus and induce PCOS in adulthood (Tata et al., 2018).

**Cortisol**

Cortisol is one of the major glucocorticoid hormones synthesised in the adrenal cortex under the control of the HPA axis. The organisation of the HPA axis is regulated by sex hormones (Handa et al., 1994; Pasquali, 2012; Viau, 2002; M. Williamson, Bingham, & Viau, 2005). Hypercortisolism or abnormally high cortisol secretion is a well-known feature of MDD and has been related to a dysfunctional hyperactive HPA axis (Pariante & Miller, 2001). Cortisol has been shown to inhibit ACTH and corticotrophin releasing factor (CRF) synthesis and release from the pituitary and the hypothalamus respectively, ultimately resulting in a drop in cortisol levels (known as the negative feedback signal). Excess cortisol may lead to Cushing’s syndrome or amenorrhoea (absence of menstrual periods during reproductive years), whereas a deficiency in cortisol levels has been related to Addison’s disease.
Androgens and cortisol have been shown to be related, with an elevated Cortisol/DHEA ratio demonstrated in depression (A. Young, Gallagher, & Porter, 2002).

3.4 NEUROBIOLOGY OF ANDROGENS

3.4.1 Important brain areas in androgen metabolism

The brain contains receptors for androgens and oestrogens (Adhya et al., 2018; Arevalo et al., 2015; Handa, Reid, & Resko, 1986; Kruijver, Balesar, Espila, Unmehopa, & Swaab, 2003; Kruijver, Fernández-Guasti, Fodor, Kraan, & Swaab, 2001; Puy et al., 1995; Simerly, Swanson, Chang, & Muramatsu, 1990; Toran-Allerand, 2004; Zuloaga, Puts, Jordan, & Breedlove, 2008a) and is known to be efficient at producing and metabolising these sex hormones (Altman, 2004; Stoffel-Wagner, 2003). Brain areas crucial for learning and memory in humans such as the hippocampus, prefrontal cortex, amygdala and the hypothalamus have abundant androgen receptors (Beyenburg et al., 2000; Finley & Kritzer, 1999; Sarrieau et al., 1990). Studies have shown that some brain areas contain the enzyme aromatase and the androgen metabolite 5α-reductase which are crucial for the conversion of testosterone to either DHT or oestrogen (Baulieu, 1998; Stoffel-Wagner, 2003). Research has increasingly demonstrated that androgens have neuromodulatory actions in the development of the brain (Do Rego et al., 2009; Janowsky, 2006; McEwen, Alves, Bulloch, & Weiland, 1998), depending upon their binding to respective androgen or oestrogen receptors. Androgen deprivation has been shown to be associated with loss of hippocampal density in ovariectomised female rats, restored with androgen treatment (Leranth, Hajszan, & MacLusky, 2004). Some evidence suggests that testosterone may be neuroprotective in both older males and females (Kurth et al., 2014; Pike, Carroll, Rosario, & Barron, 2009). Pike (1999) demonstrated that testosterone neuroprotection was not reduced by an oestrogen receptor antagonist, suggesting that testosterone together with oestrogen provide a neuroprotective ability, however, through distinct mechanisms. The effects of androgens on the brain may be classified
into two fundamentally separate categories- organisational and activational effects (Arnold, 2009; Goel & Bale, 2008; Schulz, Molenda-Figueira, & Sisk, 2009).

### 3.4.2 Organisational effects of androgens

Testosterone has differential effects on the developing male and female brain, and this androgen continues to have an effect into adulthood (Baron-Cohen, Knickmeyer, & Belmonte, 2005; Filova, Ostatnikova, Celec, & Hodosy, 2013). Androgens play a crucial role in brain organisation during early development (Goel & Bale, 2008; Swerdloff et al., 1992), which is observed in sex-typical behaviours in childhood and later in adulthood (Berenbaum, 1999; Berenbaum, Bryk, & Beltz, 2012; Hines, 2010). In foetal and neonatal stages, androgens act on the brain, and are responsible for differentiation in neural structures and function (Baron-Cohen et al., 2005). Prenatal exposure to testosterone has been shown to have permanent effects on the organisation of the brain, affecting its morphology (Weiner, Primeau, & Ehrmann, 2004). The brain regions with greatest sexual dimorphism in adulthood also have the highest expression of sex hormone receptors during brain development (J. Goldstein et al., 2001).

Animal studies also suggest that sex hormones play an important organisational role in the central nervous system, and may be associated with permanent changes in the morphology in the nervous system (Foecking, McDevitt, Acosta-Martínez, Horton, & Levine, 2008; D. Shi & Vine, 2012). Prenatal sex hormones including androgens in animals have been linked to sex differences in cognitive function into adulthood (Isgor & Sengelaub, 1998; Karaismailoğlu & Erdem, 2013). Animal studies involving the development of a female reproductive system in a castrated (therefore testosterone deprived) male rabbit foetus and the development of a male reproductive system in female animals receiving testosterone implants demonstrate the effects of testosterone on sexual characteristics (Jost, 1947; Vom Saal & Bronson, 1980). Enlargement in brain regions responsible for mating behaviour (sexually dimorphic nucleus) has been shown to be related to testosterone administration (Dominique Toran-Allerand, 1976).
Apart from sexual differentiation, testosterone has also been related to neurodevelopmental disorders affecting the human brain. Increasing evidence suggests that prenatal exposure to high concentrations of testosterone may be related to autistic spectrum disorder (ASD) (Auyeung et al., 2009; Baron-Cohen et al., 2015; Manning, Baron-Cohen, Wheelwright, & Sanders, 2001). As reported by the American Psychiatric Association (2013), ASD is a neurodevelopmental disorder characterised by impaired verbal and non-verbal communication, repetitive behaviours, and unusually restricted or stereotyped interests. This includes difficulties in social-emotional reciprocity and impairment in emotional processing. Other key symptoms involve delayed language development, reduced eye contact, and diminished ability to empathise. One biological theory to explain cognitive and emotional abnormalities in individuals with autism is the ‘Androgen Theory of Autism’ (Baron-Cohen et al., 2005).

3.4.3 Androgen Theory of Autism

The androgen theory of autism posits that autistic spectrum conditions may be associated with increased exposure to foetal or prenatal testosterone (measured in amniotic fluid) (Baron-Cohen et al., 2015; Baron-Cohen et al., 2011; Baron-Cohen & Wheelwright, 2004; Ingudomnukul, Baron-Cohen, Wheelwright, & Knickmeyer, 2007). Interestingly, high foetal testosterone levels have been found to negatively correlate with cognitive function and emotion processing (particularly empathy) in individuals with ASD (E. Chapman et al., 2006; Knickmeyer et al., 2006; Knickmeyer, Baron-Cohen, Raggatt, & Taylor, 2005). In children, higher levels of foetal testosterone have been found to negatively correlate with eye contact at 12 months of age, verbal ability (vocabulary) at 18 months and social relationships at 4 years of age (Knickmeyer et al., 2005; Lutchmaya, Baron-Cohen, & Raggatt, 2002a, 2002b). Regarding brain development, smaller corpus callosum, gyrus, lateral frontoparietal cortex and lower connectivity between hemispheres has been found to be significantly associated with higher foetal testosterone exposure (Auyeung et al., 2009).
There is a significant male bias in autistic spectrum conditions, with ratios as high as 4:1 (four affected males for every one affected female) in individuals with ASD (Baron-Cohen et al., 2011; Gillberg, Cederlund, Lamberg, & Zeijlon, 2006; Werling & Geschwind, 2013), suggesting androgens may be involved in the development of this disorder. A typical male profile has been found in the neurodevelopment of autistic spectrum conditions and some studies have suggested hyper-masculinisation in parents of offspring with autism (Baron-Cohen et al., 2005). The consistent finding of a negative association between foetal testosterone and empathy and social functioning suggest significant organisational effects (Baron-Cohen et al., 2011). Evidence shows positive associations between foetal testosterone and autistic traits in infancy and also in childhood (Auyeung, Taylor, Hackett, & Baron-Cohen, 2010). Impairment in facial emotion processing has been found in individuals with autism and together, with the male-bias of autism, evidence for this theory appears strong (Baron-Cohen et al., 2011). Females with abnormally elevated testosterone levels have been found to have a higher number of autistic traits compared with unaffected siblings (Knickmeyer et al., 2006). Additionally, studies have found a higher incidence of PCOS related symptoms including ovulatory dysfunction, hirsutism and cystic acne (related to excess androgens) (see Chapter Five for a detailed description of the syndrome) in women with autistic spectrum conditions (Ingudomnukul et al., 2007; Pohl, Cassidy, Auyeung, & Baron-Cohen, 2014). Therefore, the common risk factor, elevated testosterone levels, may be implicated in both autistic spectrum conditions and in conditions involving abnormalities in the androgen system into adulthood (Ingudomnukul et al., 2007).

There are complications in investigating the organisational effects of sex hormones since measurements before birth (prior to brain organisation) and follow-up data are difficult to obtain and involve ethical considerations. However, a putative physical marker of foetal testosterone accessible to researchers is the digit length ratio, also known as the 2D:4D ratio. The 2D:4D finger ratio is an index of the length ratio between the 2nd and 4th digit (lower ratio indicates
high foetal testosterone) (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004; Manning et al., 2001; Romero-Martínez et al., 2013). Foetal testosterone has been found to stimulate the growth of the fourth finger (starting from the thumb) whereas oestrogen has been found to promote the growth of the index finger. Males have a significantly lower 2D:4D ratio on their right hand (longer fourth finger), compared with females, which is negatively associated with prenatal testosterone (Breedlove, 2010; Hönekopp, Bartholdt, Beier, & Liebert, 2007; Lutchmaya et al., 2004). Furthermore, a lower 2D:4D ratio (high foetal testosterone) has been found to be associated with lower scores on empathy measures in individuals with autistic spectrum disorders and their parents (Manning et al., 2001; Romero-Martínez, de Andrés-García, Ruiz-Robledillo, González-Bono, & Moya-Albiol, 2014).

One study found that a single administration of testosterone in sixteen healthy women was followed by significantly impaired cognitive function, and this effect was associated with the 2D:4D ratio (van Honk et al., 2011). Moreover, the ratio predicted more than 50% variance in this effect, with a significant effect of testosterone observed in females with low ratios (higher prenatal exposure to testosterone). It has also been suggested that the digit ratio is sensitive to total androgen stimulation, related to the interaction between prenatal testosterone exposure and ambient androgen sensitivity (Breedlove, 2010), however, with mixed evidence (Hönekopp et al., 2007).

### 3.4.4 Activational effects of testosterone

Although the androgen theory of autism is related to the organisational effects of testosterone, research suggests that the activational effects of testosterone, observed in experimental studies involving testosterone administration, may also impact emotion processing in females (Bos, van Honk, Ramsey, Stein, & Hermans, 2013; Hermans et al., 2007; van Honk & Schutter, 2007; van Honk et al., 2011). Testosterone exposure in males including both the early brain formation period and puberty is important for the development of masculinisation of the neurocircuitry involved
Research suggests that adolescence and later adulthood may be a sensitive period for testosterone-dependent brain organisation. The hormonal milieu including elevated levels of sex hormones during puberty and adolescence (activational effects of testosterone), and the interaction between increased levels of hormones and the brain, may have significant effects on mood and cognitive and social function in individuals (Hermans, Putman, & Van Honk, 2006; Ingudomnukul et al., 2007; Sisk & Zehr, 2005; van Honk, 2009; van Honk & Schutter, 2007). Circulatory serum androgens may modulate brain function in later adulthood (Kimura & Hampson, 1994; van Wingen et al., 2009). Since mood disorders are more prevalent during the reproductive age in females, it has been hypothesised that sex hormones during this stage influence the neurotransmitter receptors in the brain leading to depressive symptoms (Janowsky, Halbreich, & Rausch, 1996).

Although activational effects are not responsible for permanently changing brain morphology, they modulate neurotransmitter receptors in the brain, which in turn, may affect mood and associated cognitive function (Halbreich, Lemus, Lieberman, Parry, & Schiavi, 1990; J. Hamilton, Parry, & Blumenthal, 1988; Janowsky et al., 1996; Murray, 1991; Parry, 1989; Steiner, 1987), however, this needs further investigation. Experimental studies involving testosterone administration have shown decreased recognition of consciously shown emotional faces, decreased gaze avoidance and gaze aversion and worse performance on measures of emotion processing following testosterone administration in healthy females and in females with Social Anxiety Disorder (Hermans et al., 2007; Olsson, Kopsida, Sorjonen, & Savic, 2016; van Honk & Schutter, 2007; van Honk et al., 2011) (see Chapter 5 for a review of literature). Such interventional studies implicate the need for better understanding of the effect of excess
androgens on brain function in females since androgen abnormalities may play a role in depressive symptomatology and impaired cognitive function.

### 3.5 ANDROGEN DISORDERS

Abnormalities in the androgen system may lead to excess or deficient production of testosterone in females causing endocrine disorders. Many factors affect androgen production and metabolism in women of reproductive age. As discussed earlier, a decrease in SHBG levels is associated with increased testosterone levels. Decreased SHBG levels (therefore high testosterone levels) are seen in obese individuals, patients with PCOS, hypothyroidism, and Cushing syndrome. Increased SHBG levels and lower testosterone are found in liver disease, hyperthyroidism, and individuals using corticosteroids or hormonal replacement therapy and pregnant females. Androgen levels may also decrease due to ageing and menopause (Abraham, 1974; Burger & Papalia, 2006; Davison et al., 2005; Labrie, Bélanger, Cusan, Gomez, & Candas, 1997). Androgen deficiency is seen in Turner’s syndrome (Gravholt, Svenstrup, Bennett, & Sandahl Christiansen, 1999), Addison’s disease (adrenal insufficiency of cortisol and androgen production) (Hunt et al., 2000), hypopituitarism (K. Miller et al., 2001) and cardiovascular disease (Kloner, Carson, Dobs, Kopecky, & Mohler, 2016). Androgen-excess related disorders are related to more frequently seen endocrine disorders in women of reproductive age. Hyperandrogenism can affect various tissues and organs manifesting into different clinical features such as acne, hirsutism, infertility, androgenic alopecia and virilisation (Homburg, 2009; Lizneva, Gavrilova-Jordan, Walker, & Azziz, 2016).

The main disorders of androgen excess include:

1) **Congenital Adrenal Hyperplasia:** Congenital Adrenal Hyperplasia (CAH) is a genetic disorder which involves adrenal abnormalities. In addition to testosterone excess, androgen precursors such as DHEAS and 17OH-Progesterone (17OHP) are significantly elevated (Berenbaum et al., 2012; Jacobs, Edelheit, Coleman, & Herzog, 1999; Little, 2013). CAH...
can be classified according to the specific type of enzyme deficiency involved. In classic CAH, with a more severe form of enzyme deficiency, females who may be born with ambiguous genitalia experience virilisation due to excess androgen exposure (Mueller et al., 2014). Non-classic CAH is a milder variant with less severe enzyme deficiency and has a later-onset with mild hyperandrogenism similar to PCOS and is therefore considered as a differential diagnosis to PCOS (Solomon, 2007).

2) **Cushing Syndrome**: High cortisol levels (overproduction by adrenal glands), and moderately high androgen levels (clinically observed as hirsutism and menstrual irregularities) are seen in individuals with Cushing syndrome (Barbetta et al., 2001; Haouat et al., 2012).

3) **Polycystic Ovarian Syndrome**: The most common endocrine disorder in women of reproductive age is PCOS and will be discussed in the next chapter.

### 3.6 KEY POINTS

Androgens have important actions in women's physiology. Women of reproductive age have the greatest risk of developing mood and anxiety disorders, which in some cases, may be related to abnormal androgen function. The main types of androgens in women are testosterone (Total and Free) and precursors including DHEA, DHEAS and androstenedione. Androgen receptors in women are highly expressed in brain areas responsible for cognitive function and emotion processing.

The androgen theory of autism posits that exposure to higher levels of testosterone in the foetal stage may cause autism, which involves social and emotional deficits in individuals, similar to deficits observed in MDD. Women with endocrine disorders such as PCOS (the most common endocrine disorder) have been reported to have a higher prevalence of symptoms of depression.
and anxiety and impairments in cognitive function and emotion processing; suggesting androgens may play a developmental role.
4.1 INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in females of reproductive age (Azziz, Woods, et al., 2004; Bozdag, Mumusoglu, Zengin, Karabulut, & Yildiz, 2016; Farrell & Antoni, 2010; Franks, 1995; McGowan, 2011), affecting up to 5 to 22% of females depending upon the diagnostic criteria used (Azziz et al., 2016; Bozdag et al., 2016; Farquhar et al., 1994; Goodarzi & Azziz, 2006; Knochenhauer et al., 1998; Lizneva, Suturina, et al., 2016; March et al., 2010; Mueller et al., 2014; Nestler, 1998; Sirmans & Pate, 2014; Teede et al., 2010). Polycystic ovarian syndrome involves a wide variety of clinical features, and its aetiology still remains unclear (Azziz, 2004; J. Strauss, 2003; Tsilchorozidou, Overton, & Conway, 2004). Recent evidence suggests that PCOS might be a complex multigenic disorder with substantial epigenetic and environmental influences (Escobar-Morreale, 2018). Clinical and research understanding of PCOS has evolved, from the syndrome being perceived as purely a menstrual and cosmetic problem to a multi-faceted disorder involving metabolic and hormonal abnormalities (Fenton, 2005). However, the central biochemical abnormality of PCOS is androgen (testosterone) excess (Azziz et al., 2009; Franks, 1991; Homburg, 2009). Prevalence studies have consistently documented higher rates of mood and anxiety disorders in females with PCOS (Cooney & Dokras, 2017; Dokras et al., 2012; Teede et al., 2011).

The current chapter will introduce the clinical features, epidemiology and risk factors involved in PCOS. The complexities involved in the assessment and treatment of PCOS will also be
discussed. The final part of the chapter will review findings related to studies examining the relationship between PCOS and mood and cognitive function.

4.2 CLINICAL FEATURES

4.2.1 Background

First described in 1935 as ‘Stein-Leventhal’ syndrome, the exact definition of PCOS is still contested (I. Stein, 1959). Drs Stein and Leventhal’s findings involving seven patients with oligomenorrhea (irregular menses with fewer than nine menses per year) or amenorrhea (no menses) bilaterally polycystic ovaries, obesity and hirsutism constituted the first report of the syndrome (I. Stein & Leventhal, 1935). This heterogeneous disorder, now well-known as PCOS, consists of broad and variable clinical manifestations. Cardinal features include elevated androgen levels (hyperandrogenism), observed as physical symptoms of cystic acne (Reingold & Rosenfield, 1987; Scholl, Wu, & Leyden, 1984; Slayden, Moran, Sams, Boots, & Azziz, 2001; Vexiau et al., 1990), excessive and unwanted terminal hair growth on the body (hirsutism), particularly the face, back, chest and abdominal regions (Azziz, Carmina, Dewailly, Diamanti-Kandarakis, Escobar-Morreale, Futterweit, Janssen, Legro, Norman, Taylor, et al., 2006; Diamanti-Kandarakis et al., 1999; Fauser et al., 2012; Sirmans & Pate, 2014; Spritzer, Barone, & Oliveira, 2016), androgenic alopecia (thinning scalp hair, male pattern baldness) (Cela et al., 2003), virilisation (Azziz, Sanchez, et al., 2004), chronic anovulation and ovulatory dysfunction (including menstrual disturbances) (Balen & Rutherford, 2007; Franks, 1995; Hull, 1981), and polycystic ovarian morphology (PCOM) in the form of multiple ovarian cysts (Azziz et al., 2016). Other important features are infertility (Hart, 2008; Teede et al., 2010), insulin resistance and obesity (Dunaif, Segal, Futterweit, & Dobrjansky, 1989; Teede et al., 2010; Valenzuela & Stone, 2014). Clinical symptoms are often classified into three main categories, related to abnormalities in the reproductive, hormonal, and metabolic systems, respectively.
Currently, metabolic symptoms are not formally recognised in diagnostic classification systems, but are known to be strongly related to, and highly prevalent in PCOS. The metabolic features of PCOS are thus discussed in the Risk Factors section of this chapter (see page 64).

4.2.2 Hormonal features

The main hormonal abnormality involved in PCOS is hyperandrogenism (Azziz et al., 2016). This feature may be observed as either clinical hyperandrogenism including cosmetic features such as hirsutism, acne and alopecia, and/or biochemical hyperandrogenism including androgen excess. Elevated circulating androgen levels (mainly Free Testosterone) are commonly found in patients (Balen et al., 1995; Hahn et al., 2005; Huang et al., 2010; Lujan, Chizen, & Pierson, 2008). The three main diagnostic criteria for PCOS (including Rotterdam, National Institutes of Health [NIH] and Androgen Excess and PCOS Society [AE-PCOS]; refer to page 59) include hyperandrogenaemia as a common diagnostic feature of PCOS compared with other features of PCOS (ovulatory dysfunction and ovarian cysts). High FAI levels are another important endocrine marker of symptoms involved related to ovarian function in PCOS (Imani et al., 2000; Mathur, Moody, Landgrebe, & Williamson, 1981; Vermeulen et al., 1999). Sex Hormone Binding Globulin (SHBG) levels are often lower (therefore Free Testosterone levels are higher) in females with PCOS (Jayagopal, Kilpatrick, Jennings, Hepburn, & Atkin, 2003; Pugeat, Crave, Tourniaire, & Forest, 1996). Absolute levels of circulating Luteinising Hormone (LH), and the LH/Follicle Stimulating Hormone (LH/FSH) ratio, are also significantly higher in females with PCOS (Fauser et al., 1991; Ropelato et al., 1999; A. Taylor et al., 1997). Adrenal precursor androgens including DHEA, DHEAS (Azziz, Sanchez, et al., 2004; Brennan, Huang, & Azziz, 2009; Goodarzi, Carmina, & Azziz, 2015), and A-dione levels (O'reilly et al., 2014) are higher due to ovarian and adrenal hypersecretion.
4.2.3 Reproductive features

Polycystic Ovarian Syndrome was originally understood as a condition characterised by multiple enlarged ovarian cysts observed at the time of surgery (I. Stein & Leventhal, 1935). It was thought that ovarian cysts uniquely characterised PCOS, however, in recent times, studies have concluded that PCOS is a complex syndrome, and may be present even in females with regular, ovulatory, menses (without reproductive or ovulatory dysfunction), but showing signs of hyperandrogenism (Adams, Polson, & Franks, 1986; Carmina, Koyama, Chang, Stanczyk, & Lobo, 1992; Clayton et al., 1992; Farquhar et al., 1994; Polson, Wadsworth, Adams, & Franks, 1988; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Polycystic ovarian morphology (PCOM) is characterised by an excessive number of preantral follicles in the ovary; termed as multi-follicularity (Azziz et al., 2016). Ovarian cysts are clinically defined by the presence of more than 10 cysts measuring 2 to 8mm in diameter, distributed or scattered peripherally around a dense core of stroma (connective tissues of the ovary) (Adams et al., 1986), and are detected by ultrasound or other forms of pelvic imaging. The term ‘polycystic ovaries’ adds to the diagnostic confusion since polycystic ovaries are not the main criteria for diagnosis, and are less relevant than the volume of ovarian stroma, which is more closely related to serum testosterone levels (Fratantonio, Vicari, Pafumi, & Calogero, 2005; Kyei-Mensah et al., 1996). In addition, multiple cysts on ultrasound have been detected in 20 to 30% of normal cycling non-hyperandrogenic females (Clayton et al., 1992; Farquhar et al., 1994; Michelmore, Balen, Dunger, & Vessey, 1999; Polson et al., 1988). Polycystic ovarian morphology (PCOM) is associated with normal oestrogen and progesterone levels but with high androgen and low SHBG levels, suggesting PCOM may be indicative of mild ovarian hyperandrogenism (Adams, Taylor, Crowley Jr, & Hall, 2004; Fratantonio et al., 2005). It has been recently suggested that PCOM may also be related to, and a significant predictor of, insulin resistance (refer to section 4.5.1), which is a key metabolic feature of PCOS (Hong et al., 2017). However, since clinical and biochemical features of hyperandrogenism and hyperinsulinemia
in PCOS may exist independently of ovarian cysts, there has been a shift from the original definition of PCOS to a broader, more inclusive understanding of the syndrome involving metabolic and hormonal abnormalities (Dumesic et al., 2015; Dunaif & Fauser, 2013). However, a recent study found that inclusion of ovarian morphology results in statistically significant higher prevalence estimates for PCOS (Skiba, Islam, Bell, & Davis, 2018).

Other reproductive features may present in the form of anovulation or ovulatory dysfunction. About 70 to 80% of patients with PCOS have ovarian dysfunction, which manifests as oligo or amenorrhea (Ehrmann, 2005). Prolonged anovulation may lead to dysfunctional uterine bleeding which may mimic a more regular menstrual cycle (Teede et al., 2010). As many as 85% of females with PCOS experience menstrual irregularities (Azziz et al., 2009), however, newer diagnostic criteria (see Section 4.3) consider females with regular menstrual cycles eligible for receiving diagnosis if they show hyperandrogenic or metabolic symptoms (Azziz, Carmina, Dewailly, Diamanti-Kandarakis, Escobar-Morreale, Futterweit, Janssen, Legro, Norman, Taylor, et al., 2006).

Another common reproductive feature involved in PCOS symptomatology is infertility (Hull, 1987; Nestler, 2008; Wild, Pierpoint, Jacobs, & McKeigue, 2000). The primary cause of infertility is considered to be anovulation (found in PCOS), accounting for more than 75% of cases of infertility (Gorry, White, & Franks, 2006; Joham, Teede, Ranasinha, Zoungas, & Boyle, 2015).
Table 4.1
Common Symptoms of Polycystic Ovarian Syndrome

<table>
<thead>
<tr>
<th>Reproductive</th>
<th>Hormonal</th>
<th>Metabolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Multiple ovarian cysts</td>
<td>• Biochemical abnormalities including hyperandrogenism/ androgen excess (high testosterone levels)</td>
<td>• Insulin resistance and hyperinsulinemia</td>
</tr>
<tr>
<td>• Infertility</td>
<td>• Clinical hyperandrogenism: hirsutism, cystic acne, alopecia, skin tags, acanthosis nigricans (dark velvety patches in body folds and creases).</td>
<td>• Obesity (particularly central obesity) and weight gain</td>
</tr>
<tr>
<td>• Oligo/anovulation</td>
<td></td>
<td>• Insulin resistance can lead to lipid abnormalities, impaired glucose tolerance</td>
</tr>
<tr>
<td>• Oligomenorrhea (menstrual irregularity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pelvic pain, uterine bleeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Poly-cystic ovarian morphology/multifollicularity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3 DIAGNOSIS / CLASSIFICATION

1) The National Institutes of Health (NIH) - National Institute of Child Health and Human Disease (NICHD) 1992 Criteria: Using this 1990 US criteria, PCOS can be defined by i) presence of clinical and/or biochemical hyperandrogenism, ii) chronic anovulation, and iii) absence of other endocrine disorders. The NIH diagnostic criteria do not include the presence of polycystic ovaries on ultrasound (Zawadzki, 1992) (as cited by Azziz, 2006).

2) The Rotterdam Criteria (proposed by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) expert conference held in Rotterdam: The Rotterdam criteria (2004) requires two of the three following features: i) clinical and/or biochemical hyperandrogenism (elevated Free Testosterone and FAI levels, reduced SHBG levels, increased DHEAS levels, hirsutism) (Welt et al., 2006; Zawadzki, 1992); ii) oligo or anovulation, and iii) polycystic ovaries and exclusion of other endocrinopathies. Under this criteria, new phenotypes of PCOS emerge, for example, patients with polycystic ovaries and hyperandrogenism but with normal ovulation, or alternatively, patients with anovulation and cystic ovaries without clinical or biochemical androgen excess (Azziz, 2004). One of the main objectives of the Rotterdam criteria was to correct for the increasing evidence related to clinical and biochemical hormonal features in females with PCOS, making these criteria broader than the original NIH diagnostic criteria. An expert panel from the NIH Evidence-Based Methodology Workshop on PCOS recommended that clinicians use the Rotterdam diagnostic criteria for PCOS since it is more recent and relevant compared with the NIH criteria (Dumesic et al., 2015; Lizneva, Suturina, et al., 2016).

3) Androgen Excess and PCOS (AE-PCOS) Society

The more recent AE-PCOS criteria (Azziz, Carmina, Dewailly, Diamanti-Kandarakis, Escobar-Morreale, Futterweit, Janssen, Legro, Norman, & Taylor, 2006) defines PCOS as
involving the presence of clinical or biochemical hyperandrogenism (including hirsutism and conditions related to hyperandrogenemia) and ovulatory dysfunction (oligo-anovulation or polycystic ovaries) (Azziz et al., 2009), however, emphasising hyperandrogenism. Females with PCOM and oligo-anovulation (without androgen excess) do not meet the criteria for a diagnosis of PCOS based on AE-PCOS Society (Azziz, Carmina, Dewailly, Diamanti-Kandarakis, Escobar-Morreale, Futterweit, Janssen, Legro, Norman, Taylor, et al., 2006).

Both the AE-PCOS and the Rotterdam Criteria definitions represent broad extensions of the original NIH criteria. Since the diagnosis of PCOS is based on exclusion, all three criteria ensure omission of differential diagnosis or other endocrinological conditions and ovulatory dysfunction which may resemble PCOS symptomatology. These include: 1) thyroid dysregulation, 2) hyperprolactinemia, 3) pregnancy, 4) Cushing’s syndrome, 5) androgen-producing tumours, 6) drug-induced androgen excess and, 7) late-onset Congenital Adrenal Hyperplasia (Azziz et al., 2009; Dumesic et al., 2015; Franks, 1995; Yildiz, Bozdag, Yapici, Esinler, & Yarali, 2012; Zawadzki, 1992). Diagnostic criteria for PCOS has been developed by three groups (see Table 4.2).
### Diagnostic Criteria for Polycystic Ovarian Syndrome

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Hyperandrogenaemia</td>
<td>Two of the three following criteria:</td>
<td>Both criteria to be satisfied:</td>
</tr>
<tr>
<td>• Ovulatory dysfunction</td>
<td>• Hyperandrogenaemia</td>
<td>• Hyperandrogenaemia</td>
</tr>
<tr>
<td></td>
<td>• Ovulatory dysfunction</td>
<td>• Either ovarian dysfunction, polycystic ovaries</td>
</tr>
<tr>
<td></td>
<td>• Polycystic ovaries</td>
<td>or both</td>
</tr>
</tbody>
</table>
4.4   EPIDEMIOLOGY

4.4.1   Background

Worldwide prevalence of PCOS ranges from 5 to 22% depending upon the diagnostic criteria used (Azziz et al., 2016). Although the number of females diagnosed with PCOS is significantly increasing (Ganie & Kalra, 2011), the reported prevalence rate may still not be entirely accurate due to being diagnosed less frequently (simply attributed to obesity) or misdiagnosed in clinical practice (Christensen et al., 2013; Sirmans, Parish, Blake, & Wang, 2014). Additionally, it has been thought that although it is likely that PCOS affects females at all ages, symptoms of hyperandrogenism may be more visible and better able to be clinically evaluated during reproductive age. Therefore, symptoms of hyperandrogenism and ovulatory function may be only weakly visible at each end of the reproductive spectrum (Goodarzi et al., 2015).

One retrospective study found that estimated prevalence rates changed along with different diagnostic criteria being used (which increased using the Rotterdam criteria) (March et al., 2010). Regarding symptomatology involved in PCOS, approximately 85 to 90% of females with irregular menses may be diagnosed with PCOS, while 30 to 40% of females with amenorrhea have been found to be at risk of developing PCOS (Hart, 2008; Sirmans & Pate, 2014). Over 80 to 90% of normally menstruating females with cutaneous hyperandrogenic symptoms are routinely diagnosed with PCOS using ultrasound measures (Adams et al., 1986; Azziz, Sanchez, et al., 2004).

4.4.2   Cross-Cultural Differences

The overall prevalence rate (5 to 22%) of PCOS in females is largely derived from studies involving European females with the syndrome (Farquhar et al., 1994; K. Williamson, Gunn, Johnson, & Milsom, 2001). However, studies have shown that racial origin and ethnicity significantly influence PCOS symptomatology (Ehrmann et al., 2002; K. Williamson et al.,
One study in New Zealand found a strikingly high prevalence of PCOS (52%) in Indian subcontinent Asian females (Rodin, Bano, Bland, Taylor, & Nussey, 1998). Dunaif et al. (1993) found a higher prevalence of PCOS in Hispanic females compared with Caucasian females. Similarly, considerable differences in symptomatology related to ethnicity were found in one cross-sectional study involving European, Maori, Indian, Chinese, Asian and Pacific Island females found (K. Williamson et al., 2001). A higher incidence of hirsutism was observed in European and Maori females compared with other ethnic groups. Acne was rarely observed in Pacific Island females, however, infertility was commonly reported in this group along with Indian females compared with European females with PCOS. Furthermore, non-European females (Indian, Pacific Island and Maori females) had the greatest rate of obesity, insulin resistance and lipid abnormalities, possibly due to an influence of a western lifestyle involving diet and lifestyle factors (Hodge et al., 1994), which are implicated in PCOS.

4.4.3 Cultural differences in prevalence related to obesity and metabolic features

Carmina et al. (1992) found a comparable prevalence of ovarian cysts (68 to 80%), testosterone levels, DHEAS levels, and insulin resistance, between Japanese and Caucasian females with PCOS. However, Japanese females were found to have less hirsutism, lower obesity and insulin levels (but not insulin resistance). Authors concluded that obesity and hyperandrogenism are affected by dietary, environmental and ethnicity-related factors. Other studies have varied ethnic groups including African-American, Indian, Mexican-American and South-Asian females with PCOS with comparable BMI, to have higher incidence of insulin resistance compared with Caucasian females with PCOS, with lower insulin resistance (Dunaif et al., 1993; Ehrmann et al., 2002; Kauffman, Baker, DiMarino, Gimpel, & Castracane, 2002; Norman, Mahabeer, & Masters, 1995; Wijeyaratne, Balen, Barth, & Belchetz, 2002).
4.5 RISK FACTORS

PCOS involves multiple risk factors including obesity, environmental and lifestyle-related factors, which contribute to the complex presentation of the disorder (including the metabolic abnormalities observed) in the clinical picture and pose a diagnostic challenge. No single aetiological factor is sufficient to fully explain the varied range of symptoms in individuals with PCOS. Studies have shown genetic, metabolic, and environmental factors to play a synergistic role in the development of the syndrome (Dumesic et al., 2015).

4.5.1 Metabolic risk factors: Insulin resistance

Polycystic Ovarian Syndrome is associated with increased risk for metabolic disorders (G. Conway et al., 2014; Sirmans & Pate, 2014). Pathogenic metabolic factors such as insulin resistance with compensatory hyperinsulinemia are risk factors for PCOS and put individuals with PCOS at risk for lipid abnormalities, impaired glucose tolerance and Type 2 diabetes. (Apridonidze, Essah, Iuorno, & Nestler, 2005; Chang, Nakamura, Judd, & Kaplan, 1983; Ciaraldi et al., 1992; Diamanti-Kandarakis & Dunaif, 2012; Dunaif, 1997; Futterweit, 1999; Goudas & Dumesic, 1997; Tsilchorozidou et al., 2004). Hyperinsulinemia is, however, not formally recognised in current diagnostic classification systems, as noted in the first section of this chapter (Azziz, 2006). Insulin resistance or hyperinsulinemia is defined as a decreased ability of insulin to regulate the metabolic actions of glucose uptake, production or lipolysis, which further leads to elevated insulin levels relative to glucose levels (Bergman, Finegood, & Ader, 1985; Dunaif & Finegood, 1996; Dunaif et al., 1992; Hardy, Czech, & Corvera, 2012; Kahn, 1985; Ovalle & Azziz, 2002). Although not a diagnostic requirement, insulin resistance is present in most females with PCOS, to some degree (Balen & Michelmore, 2002; Barber, Wass, McCarthy, & Franks, 2007; Dokras et al., 2005; Dunaif, 1997; Dunaif et al., 1989; Farrell & Antoni, 2010; Legro, Kunselman, Dodson, & Dunaif, 1999). Although insulin resistance plays a crucial role in exacerbating obesity, some studies have shown insulin resistance to be...
independent of obesity (Diamanti-Kandarakis & Dunaif, 2012; Dunaif et al., 1989; Stepto et al., 2013). Obesity is well-known as a significant risk factor for PCOS and associated disorders in females.

Strong associations between PCOS and metabolic syndrome including central obesity and dyslipidaemia have been documented (Rubin, Glintborg, Nybo, Abrahamsen, & Andersen, 2017; Sharpless, 2003). A significant proportion of females with PCOS have been shown to be overweight or obese (Álvarez-Blasco, Botella-Carretero, San Millán, & Escobar-Morreale, 2006; Isikoglu, Berkkanoglu, Cemal, & Ozgur, 2007; S. Lim, Davies, Norman, & Moran, 2012; Moran, Hutchison, Norman, & Teede, 2011), which may further exacerbate associated metabolic and reproductive abnormalities such as infertility (Brassard, AinMelk, & Baillargeon, 2008). Weight gain is associated with worsening of symptoms while weight loss has been shown to help improve the metabolic and endocrinal profile and associated symptoms in females with PCOS (Teede et al., 2013). Further hormonal complications including suppressed SHBG levels (associated with increased testosterone levels) have been found in obese females with PCOS and hirsutism (Franks, 1989; Kiddy et al., 1990). In addition, metabolic abnormalities may further lead to increased risk of obstetric complications including pregnancy-induced hypertension, preeclampsia and gestational diabetes (Bjercke et al., 2002) (cited by (Azziz, Marin, Hoq, Badamgarav, & Song, 2005)).

In summary, metabolic abnormalities associated with PCOS may lead to the development of Type 2 diabetes, glucose intolerance, hyperlipidaemia, and a risk of cardiovascular disease, hypertension and endometrial hyperplasia (Daniilidis & Dinas, 2009; Legro, 2003; Wild, 2002). Androgen excess has been thought to play a pivotal role in exacerbating these conditions (Apridonidze et al., 2005; Chen et al., 2007; Coviello, Legro, & Dunaif, 2006; Diamanti-Kandarakis, Papavassiliou, Kandarakis, & Chrousos, 2007; Korhonen, Hippeläinen, Vanhala, Heinonen, & Niskanen, 2003), since correcting androgen abnormalities can help insulin
sensitivity and improve metabolic profile (Dahlgren, Landin, Krotkiewski, Holm, & Janson, 1998).

4.5.2 Genetic risk factors

Genetic studies have attempted to identify genes that contribute substantially to the development of the PCOS phenotype, however, without compelling evidence (De Leo et al., 2016; Diamanti-Kandarakis, Kandarakis, & Legro, 2006; Franks, Gharani, & McCarthy, 2001; Franks, McCarthy, & Hardy, 2006; Kosova & Urbanek, 2013; Mutharasan et al., 2013; Shen et al., 2013; Xu et al., 2011). Nevertheless, family history is a substantial risk factor for PCOS (Amato & Simpson, 2004; Battaglia et al., 2002; Crosignani & Nicolosi, 2001; Franks et al., 1997; Franks et al., 2008; Givens, 1988; Hague, Reeders, Peto, & Jacobs, 1988; Kahsar-Miller, Nixon, Boots, Go, & Azziz, 2001; Legro, Bentley-Lewis, Driscoll, Wang, & Dunaiif, 2002; Vink, Sadrzadeh, Lambalk, & Boomsma, 2006). Family studies have found sisters of females with PCOS to have higher than normal androgen levels (Hong et al., 1998; Legro, Driscoll, Strauss, Fox, & Dunaiif, 1998) and monozygotic twins to be more affected with PCOS than dizygotic twins (Vink et al., 2006); suggesting genetic involvement in the syndrome.

Animal studies have suggested that excess foetal androgen exposure in female non-human primates induces PCOS-like symptoms (Abbott, Dumesic, Eisner, Kemnitz, & Goy, 1997; Padmanabhan, Manikkam, Recabarren, & Foster, 2006) via alteration of the epigenome (chemical modifications to the DNA which alter gene expression) (Z. Li & Huang, 2008; Xu et al., 2011). Hyperandrogenism has been found to induce the epigenetic alterations of genetic components in ovarian granulosa cells that are involved in the ovulatory dysfunction aspect of PCOS (Qu et al., 2012). However, there are mixed findings in the literature, and the single-gene aetiology hypothesis has been rejected by several genome-wide association studies (De Leo et al., 2016). However, these studies show evidence consistent with PCOS having a polygenic component (Dumesic et al., 2015) with significant novel loci (Chen et al., 2011; Hayes et al.,
implicating gonadotropin and gonadotropin receptor variants to be associated with PCOS phenotypes (Azziz, 2016; Dumesic et al., 2015). Since the PCOS phenotype is varied and complex, it has been concluded that the diverse clinical presentation may result from its aetiological heterogeneity which needs to be further investigated.

4.5.3 Environmental risk factors

Environmental risk factors are increasingly being understood to play an important role in PCOS (Diamanti-Kandarakis, Piperi, et al., 2006). Symptoms usually appear around menarche (Franks, 2002), and may be precipitated by diet and lifestyle factors causing weight gain (Moran et al., 2011). Additionally, exposure to environmental toxins and endocrine disruptors may contribute to the development of symptoms (Kandaraki et al., 2011; Zhang et al., 2014). Environmental factors such as psychotropic medication, particularly sodium valproate (used to treat bipolar disorder and epilepsy) have been implicated in developing hyperandrogenism, obesity and ovulatory dysfunction (Franks et al., 2001; Franks et al., 1997; Isojarvi, Laatikainen, Pakarinen, Juntunen, & Myllyla, 1993; McIntyre, Mancini, McCann, Srinivasan, & Kennedy, 2003).

4.6 BIOLOGICAL MECHANISMS

Four main proposed hypotheses related to laboratory findings have attempted to explain the complex pathophysiological mechanism of the syndrome (Matalliotakis, Kourtis, Koukoura, & Panidis, 2006):

1) The LH hypothesis - A primary neuroendocrine defect further leads to increased LH production (pulse frequency and amplitude), resulting in ovarian hyperandrogenism and anovulation (Goudas & Dumesic, 1997; Insler & Lunenfeld, 1991; Yen, Vela, & Rankin, 1970).
2) **The insulin hypothesis** - Since most females with PCOS are resistant to insulin, irrespective of BMI, the pancreas compensates for insulin resistance by over-production and release of insulin (Nestler, 1998; Nestler et al., 1991; Pugeat & Ducluzeau, 1999; Weaver et al., 1990; Yki-Järvinen, Mäkimattila, Utriainen, & Rutanen, 1995), further stimulating testosterone production and decreasing SHBG levels (Adashi et al., 1981; Azziz et al., 2003; Chang et al., 1983; González, 2012; Kirschner et al., 1990; Legro, Gnatuk, Kunselman, & Dunai, 2005; Panidis et al., 1998; Panidis, Skiadopoulos, Roussou, Ioannides, & Panidou, 1995; Song, Rhodes, Veldhuis, & Butler, 2003; Valenzuela & Stone, 2014; Willis, Mason, Gilling-Smith, & Franks, 1996). In PCOS, insulin resistance is characterised by adrenal and ovarian tissue sensitivity (Diamanti-Kandarakis & Papavassiliou, 2006). Insulin resistance is the main cause of hyperinsulinemia in PCOS, which is characterised by excess levels of insulin relative to glucose (Tsilchorozidou et al., 2004), however, hyperinsulinemia is further responsible for insulin resistance (Shanik et al., 2008). Hyperinsulinemia stimulates androgen production from sources including ovarian, adrenal and adipose tissue while suppressing SHBG secretion from the liver leading to excess androgen levels observed in PCOS, further associated with anovulation.

3) **The ovarian hypothesis** - This hypothesis is linked to the LH hypothesis discussed above. An abnormal ovarian response to gonadotropin action may be secondary to androgen excess (Gilling-Smith, Willis, Beard, & Franks, 1994). A dysfunctional ovarian hyper-responsiveness to gonadotropin action has been thought to be the main underlying fault of androgen excess (Rosenfield, 1999). Evidence for this hypothesis may also be observed in the form of follicular dysfunction (including the presence of small follicles) by pelvic ultrasound measures. Studies have indicated that follicular cells may also respond to FSH stimulation implying blocked FSH activity at the ovarian level (Loh, Wang, & Matthews, 2002; Magoffin, 1989; Pang, Softness, Sweeney III, & New, 1987). Follicle Stimulating hormone levels have been linked to chronic anovulation in females with PCOS (Matalliotakis et al., 2006).
In summary, a comprehensive overview of all three hypotheses further leads to an understanding that hyperandrogenism and anovulation are the main outcomes of abnormalities involving increased LH production, increased insulin levels, increased androgen production, and decreased/blocked FSH activity (see Figure 4.2).

Elevated circulating androgen levels are further associated with physical symptoms of PCOS mainly in the form of hirsutism and acne (Azziz, Carmina, Dewailly, Diamanti-Kandarakis, Escobar-Morreale, Futterweit, Janssen, Legro, Norman, & Taylor, 2006; Escobar-Morreale et al., 2011; Hatch, Rosenfield, Kim, & Tredway, 1981; Martin et al., 2008; Sachdeva, 2010). Testosterone and DHT together exert their effect on androgen receptors and hair follicles, which in turn leads to coarse terminal hair growth (Azziz et al., 2016) mainly on the face, back, chest and abdominal region.

![Proposed pathophysiology of Polycystic Ovarian Syndrome](Jayasena & Franks, 2014)

**Figure 4.2**

Proposed pathophysiology of Polycystic Ovarian Syndrome (Jayasena & Franks, 2014)
4.7 TREATMENT

4.7.1 Background

In the early 1930s, the primary form of treating PCOS involved ovarian wedge resection to restore ovarian function and menstrual regularity (I. Stein, 1959, 1965; Wallach, Donesky, & Adashi, 1995). In later years, laparoscopic ovarian drilling was considered an alternative to ovarian surgery for treating ovulatory dysfunction (Al-Fadhli & Tulandi, 2004; Farquhar, Lilford, Marjoribanks, & Vandekerckhove, 2007; Hashim, Al-Inany, De Vos, & Tournaye, 2013; Palomba, Zullo, Diamanti-Kandarakis, & Orio Jr, 2007). However, ovarian drilling may not be an optimal treatment for PCOS, since little research has shown the effect of ovarian drilling on suppressing androgen production. Since the main hormonal abnormality in PCOS is elevated testosterone levels, the focus now tends to be on lowering elevated levels of androgens.

4.7.2 Investigations

Currently, in clinical practice, routine investigations include:

1. Hormonal measures of oligo-anovulation including LH, FSH, progesterone and oestradiol levels.
2. Hirsutism and acne score.
3. Main assays include serum testosterone levels (Total Testosterone, Free Testosterone and FAI levels) (Fenton, 2005; Hahn et al., 2007; Huang et al., 2010).
4. Additional measures may include cortisol, thyroid function, and prolactin depending on the patient’s symptoms.
5. Fasting glucose levels along with the oral glucose tolerance test are used to assess adiposity and obesity.
6. Measures of A-dione and DHEAS in some cases are recommended.
7. Serum levels of AMH may be raised in patients with PCOS and may be an additional measure based on the clinician’s recommendation.

4.7.2.1 Therapeutic targets

1) Treating hyperandrogenemia - Symptoms of hyperandrogenism (hirsutism, acne, and alopecia) are usually self-treated by individuals by seeking cosmetic assistance, for example, shaving, waxing, bleaching and electrolysis (Legro et al., 2013; Moghetti & Toscano, 2006; Sanchez, Perez, & Azziz, 2002; Trüeb, 2002). Treatment for biochemical androgen excess may involve oral contraceptive pills (Sachdeva, 2010) or anti-androgen medication (Calaf et al., 2007).

2) Improving insulin sensitivity and metabolic abnormalities - Treatment of metabolic abnormalities involved in PCOS aims to correct insulin resistance and associated symptoms of Type 2 diabetes and mainly consists of Metformin while monitoring glucose levels. Diet and lifestyle changes are most beneficial to achieve weight loss, to normalise hyperlipidaemia (Badawy, Aal, & Abulatta, 2009; Fenton, 2005; Jayasena & Franks, 2014; Markle, 2001; Sheehan, 2004) and to improve metabolic abnormalities, mood, fertility and ovulatory function, and thus, are often preferred as first-line treatment in PCOS (Kiddy et al., 1992; Moran et al., 2011; Teede et al., 2011; Thomson et al., 2010). Diet and lifestyle interventions are especially effective when used in conjunction with standard clinical treatment (Badawy & Elnashar, 2011; Hart, Hickey, & Franks, 2004).

3) Correcting anovulation and reducing infertility - Treatment for ovulatory dysfunction aims to regularise the menstrual cycle and improve fertility, and consists of anti-oestrogen medication. Anovulation is associated with low FSH concentrations and is usually treated with medications such as Clomiphene citrate, Tamoxifen, aromatase inhibitors, Metformin, glucocorticoids or gonadotropins (Badawy et al., 2009; Carroll & Palmer, 2001; Harborne,
Sattar, Norman, & Fleming, 2005; Homburg, 2005; Messinis, 2005; Nugent, Vandekerckhove, Hughes, Arnot, & Lilford, 2000; Palomba et al., 2005; Sastre, Prat, Checa, & Carreras, 2009). First-line treatment for infertility in PCOS involves Clomiphene citrate, (a selective oestrogen receptor regulator), Letrozole (aromatase inhibitor) or Metformin (Legro et al., 2014).

4.7.2 Pharmacological interventions

Pharmacological agents are used in severe cases of metabolic, reproductive and hyperandrogenic abnormalities and when lifestyle modifications fail to treat these symptoms. These include oral contraceptive pills and anti-androgen medication.

4.7.2.2 Oral contraceptive pills

Oral contraceptives (OCPs) are often used as a first-line pharmacological treatment in females with no desire to conceive (Badawy & Elnashar, 2011; Givens, Andersen, Wiser, & Fish, 1974; Legro et al., 2013; Wild, Umstot, Andersen, & Givens, 1982). Oral contraceptive pills are effective in regulating menstrual cycles and reducing ovarian androgen production. They are generally used in the form of oestrogen-progesterin combination therapy (consisting of both oestrogen and progesterone) (Ehrmann, 2005; Huber & Walch, 2006; Markle, 2001; Sirmans & Pate, 2014). Oestrogen in OCPs reduces hyperandrogenism by increasing direct negative feedback on LH secretion, stimulating SHBG production, decreasing ovarian androgen synthesis and adrenal androgen secretion (Azziz et al., 2016; Badawy & Elnashar, 2011). Progestins in OCPs inhibit the conversion of testosterone to DHT and binding of DHT to androgen receptors (Azziz, 1999; Mathur, Levin, & Azziz, 2008).
4.7.2.2.2 Anti-androgen medication

If hyperandrogenism still persists after use of the OCP then anti-androgen treatment is prescribed (Dumesic et al., 2015; Martin et al., 2008). Anti-androgen medication aims to decrease androgen production or inhibit androgen-binding receptors (Escobar-Morreale et al., 2011; Falsetti, Gambera, Platto, & Legrenzi, 2000; Koulouri & Conway, 2008; Swiglo et al., 2008; Townsend & Marlowe, 2004; Van Zuuren & Fedorowicz, 2015; Venturoli et al., 1999; Yildiz, 2008). Anti-androgen agents include Cyproterone Acetate (CPA), Spironolactone, Flutamide or 5-α-reductase activity inhibitors such as Finasteride and are helpful with managing acne, hirsutism or alopecia. Combination therapy of anti-androgens and OCPs is used in females (particularly sexually active females) with severe hirsutism or if the OCP fails to achieve desired results. Additionally, anti-androgen medication used in combination with Metformin has beneficial effects on metabolic symptoms involved in PCOS (Fenton, 2005).

4.7.2.2.3 Cyproterone Acetate

Cyproterone Acetate (CPA) is an androgen blocker, and a progestogen with anti-androgenic properties, and is commonly used to treat hyperandrogenism (Azziz, Carmina, & Sawaya, 2000; Neumann et al., 1970). Cyproterone Acetate inhibits the binding of testosterone and its conversion product 5a-dihydrotestosterone to androgen receptors (Venturoli et al., 1999). This anti-androgen is usually prescribed in doses ranging from 50 to 100mg in conjunction with Ethinyl Estradiol 20 to 50μg as an oestrogen replacement (to regularise the menstrual cycle). It is generally well-tolerated, though it is not devoid of side effects including nausea, headaches, breast tenderness and weight gain among others. However, it is considered beneficial to treat hirsutism, acne and alopecia in females with PCOS (Badawy & Elnashar, 2011).
4.7.2.2.4 Flutamide

Flutamide is an anti-androgen with similar androgen-receptor blocking effects to CPA, however, without the progestogenic effect or the effect on hypothalamic activity shown by CPA. Generally prescribed in doses ranging from 125 to 500mg daily, it is a useful medication for treating hirsutism (Escobar-Morreale et al., 2011; Koulouri & Conway, 2008).

4.7.2.2.5 Spironolactone

Spironolactone is another commonly used anti-androgen and reduces hirsutism scores by approximately 40% (Badawy & Elnashar, 2011; Lobo, Shoupe, Serafini, Brinton, & Horton, 1985; Lumachi & Rondinone, 2003; Moghetti, Tosi, et al., 2000). This anti-androgen works by being a competitive inhibitor of androgen receptor binding and suppresses LH and 5-α-reductase activity (Swiglo et al., 2008; Van Zuuren & Fedorowicz, 2015). It is generally well-tolerated in usual doses ranging from 100 to 200mg daily. Spironolactone is often used in conjunction with OCPs (Spritzer, Lisboa, Mattiello, & Lhullier, 2000), and has been found to be more effective compared with Metformin to reduce hirsutism and androgen levels (Alpanes, Alvarez-Blasco, Fernandez-Duran, Luque-Ramirez, & Escobar-Morreale, 2017).

4.7.2.2.6 Metformin

Metformin is used to reduce and manage the complications of insulin resistance involved in PCOS (Azziz et al., 2016). This medication improves peripheral insulin sensitivity (Moghetti, Castello, et al., 2000; Sam & Dunaif, 2003; Tang, Lord, Norman, Yasmin, & Balen, 2012) and in turn may decrease androgen levels (Badawy & Elnashar, 2011). Metformin has been shown to have optimal results when used in conjunction with lifestyle intervention (Naderpoor et al., 2015). Metformin also improves hyperandrogenism; although to a small extent (Naka et al., 2011) and is usually prescribed in doses ranging 1500 to 2000mg daily.
4.8 POLYCYSTIC OVARIAN SYNDROME AND MOOD

4.8.1 Background

A 2006 review stated that although more than 2,100 PubMed citations on PCOS appeared since 2000, only three percent addressed psychological concerns in females with PCOS (Himelein & Thatcher, 2006b). However, in the past decade, more studies have addressed the psychological aspect of PCOS (Cooney & Dokras, 2017; Cooney et al., 2017; Dokras, 2012; Glowinska, Zielona-Jenek, Pawelczyk, & Banaszewska, 2016; Karjula et al., 2017; Teede et al., 2010). It is now well-known that PCOS is strongly linked to mood and anxiety disorders, with consistent evidence, including meta-analyses, showing depression and anxiety levels to be higher and more severe in females with PCOS compared with healthy controls, independent of obesity and other physical aspects of the syndrome (Barry, Kuczmiereczyk, & Hardiman, 2011; Bruce-Jones, Zolese, & White, 1993; Cooney & Dokras, 2017; Cooney et al., 2017; Dag et al., 2017; Deeks, Gibson-Helm, & Teede, 2010; Dokras, 2012; Dokras et al., 2011, 2012; Elsenbruch et al., 2003; Farrell & Antoni, 2010; Kerchner, Lester, Stuart, & Dokras, 2009; Özenl et al., 2009; Veltman-Verhulst, Boivin, Eijkemans, & Fauser, 2012) (findings from these studies will be discussed in detail in the section 4.8.2). Other psychiatric illnesses including bipolar disorder (Rasgon et al., 2005; Wooderson, Gallagher, Watson, & Young, 2015), borderline personality disorder (BPD) (Roepke et al., 2010; Trisno, Worsley, & Kulkarni, 2016), body dissatisfaction and eating disorders (Bernadett & Szeman, 2016; Himelein & Thatcher, 2006a, 2006b), and poor health-related Quality of Life (QoL) (Barnard, Ferriday, et al., 2007; Ching, Burke, & Stuckey, 2007; Coffey & Mason, 2003; Crete & Adamshick, 2011; Elsenbruch et al., 2003; Jones, Hall, Balen, & Ledger, 2008; McCook, Reame, & Thatcher, 2005) are also more frequently reported in individuals with PCOS compared with healthy females. There is an overall consensus that females with PCOS are at an increased risk of psychiatric disorders and distress, and in a recent review and meta-analysis, it was found that females with PCOS show a high prevalence of depression (OR: 3.78; 95% CI: 3.03-4.72), with a moderate-to-severe degree of severity (OR:}
4.18. 95% CI: 2.68-6.52) (Cooney et al., 2017) (see Table 4.3). The higher prevalence of depression and anxiety is an important issue to address since a seven-fold increased risk, for suicide attempts was found in females with PCOS compared with controls (Mansson et al., 2008), which warrants more attention to the mental health component related to the syndrome.

Polycystic Ovarian Syndrome has been poetically described as a ‘thief of womanhood’ (Kitzinger & Willmott, 2002) because of its association with troublesome symptoms such as hirsutism, acne, alopecia, oiliness of the skin, seborrhoea (scaly and red patches on scalp) and infertility (Dumesic et al., 2015; Ferriman & Gallwey, 1961; Hahn et al., 2005; Himelein & Thatcher, 2006b; Jones et al., 2008; Mechanick & Dunaif, 1990). These symptoms may have a profound negative impact on the patient and may threaten her feminine identity, which may lead to body dissatisfaction (Himelein & Thatcher, 2006a) and may further lead to depression (Karjula et al., 2017). Studies have found associations between hyperandrogenic and metabolic aspects of the syndrome and depression and anxiety (Borghi et al., 2017; Deeks et al., 2010; Ekbäck, Lindberg, Benzein, & Årestedt, 2013; Farrell & Antoni, 2010; Naqvi et al., 2015; Scott et al., 2008). Hyperandrogenic symptoms of PCOS (for example hirsutism) have been shown to be associated with diminished QoL (Kitzinger & Willmott, 2002; Z. Li et al., 2011; Pate, 2016) and social anxiety (Sonino, Fava, Mani, Belluardo, & Boscaro, 1993). In a systematic review and meta-analysis assessing QoL in females with PCOS, it was found that the PCOS group ($n = 423$) had lower scores on all Short-Form-36 (SF-36) dimensions related to health-related QoL, compared with controls ($n = 285$), particularly in emotional role function (Z. Li et al., 2011). However, there is mixed evidence, with some studies showing no significant relationship between hirsutism and mood (Barth, Catalan, Cherry, & Day, 1993; Rahiminejad et al., 2014; Shulman, DeRogatis, Spielvogel, Miller, & Rose, 1992), which suggests that other factors such as biochemical abnormalities and obesity may also be associated with psychological abnormalities in PCOS.
4.8.2 Findings from meta-analytical reviews and other studies

Significantly increased prevalence and increased risk for symptoms of depression and anxiety has been found in females with PCOS compared with healthy females, and findings from these studies will be discussed in detail in the following section (Barry, Kuczmierczyk, et al., 2011; Blay, Aguiar, & Passos, 2016; Cooney & Dokras, 2017; Dokras, 2012; Dokras et al., 2011, 2012; Farrell & Antoni, 2010; Veltman-Verhulst et al., 2012).

A systematic review (including 17 studies) and meta-analysis (including 10 cross-sectional studies) reported a four-fold increased risk for abnormal depression scores in females with PCOS (4.03 [95% confidence interval [CI] 2.96–5.5, \( p < 0.01 \)) \((n = 522)\) compared with healthy females \((n = 475)\) across several countries, independent of BMI and the screening tool used \((\text{OR} 4.09, 95\% \text{ CI} 2.62–6.41, p < 0.01)\) (Dokras et al., 2011). Another systematic review \((n = 9\) studies) and meta-analytical study \((n = 4)\) by the same research group examined anxiety in females with PCOS. Prevalence of GAD and overall symptoms of anxiety were found to be significantly higher in females with PCOS \((n = 450)\) compared with healthy control females \((n = 377)\) \((\text{OR} 6.88, 95\% \text{ CI} 2.5-18.9, p < 0.01)\) (Dokras et al., 2012). In a recent meta-analysis (six studies) (Blay et al., 2016) anxiety \((\text{OR} =2.76; 95\% \text{ CI} 1.26\text{ to }6.02; \text{Log OR} =1.013; p = 0.011)\) and depression levels \((\text{OR} =3.51; 95\% \text{ CI} 1.97\text{ to }6.24; \text{Log OR} =1.255; p < 0.001)\) were found to be significantly higher in females with PCOS (unspecified number of females) compared with healthy control females. Despite including a stricter inclusion criterion, including studies only if they involved standardised interviews or screening tools for psychiatric evaluation and for diagnosis of PCOS (e.g., Rotterdam, NIH or AE-PCOS society criteria) symptoms of anxiety and depression were found to be higher in a sample with PCOS. Barry et al. (2011) found significantly higher depression \((Z = 17.92, p < 0.00001; \text{Hedges’ } g = 0.82; 95\% \text{ CI} 0.73–0.92)\) and anxiety levels \((Z = 5.03, p < 0.00001; \text{Hedges’ } g = 0.54; 95\% \text{ CI} 0.33–0.75)\) in their systematic review and meta-analysis, which included a total of twelve comparative studies, of which six assessed anxiety (208 PCOS, 169 healthy controls), whereas
all twelve assessed depression (910 PCOS, 1347 healthy controls). Body Mass Index was proposed to play an important role since studies controlling for BMI showed smaller differences on depression and anxiety scores in females with PCOS compared with healthy controls. However, levels of depression and anxiety were found to be only mildly elevated in females with PCOS. Similar levels of mild-moderate depression and anxiety were found in another comprehensive meta-analytical study (Veltman-Verhulst et al., 2012). Although twenty-six of twenty-eight studies found higher depression levels and seventeen studies found higher anxiety levels in females with PCOS compared with controls in this study (2012), females with PCOS (\(n = 2834\)) were found to show mild-moderate severity of depression, with half of the studies in the meta-analysis showing scores in the non-clinical range compared with healthy controls (\(n = 2705\)). Mood and anxiety symptoms were found to be similar in all phenotypes of PCOS, for example, obese females with infertility and hirsutism had similar depressive levels compared with lean fertile females.

A recent systematic review (including 30 cross-sectional studies) and meta-analysis (18 studies assessing depression and 9 studies assessing anxiety) found females with PCOS (\(n = 3050\)) to have significantly increased odds of both moderate and severe mood and anxiety scores compared with healthy controls (\(n = 3858\)) (Cooney & Dokras, 2017). Females with PCOS had over three times the odds of moderate-severe symptoms of depression and over five times the odds of symptoms of anxiety compared with healthy controls. In the meta-regression analysis (based on 15 studies separate from the systematic review), females with PCOS and depression had higher mean values of age, BMI, hirsutism and insulin resistance (but not testosterone levels) and females with PCOS and anxiety showed higher mean values of BMI, hirsutism and Free Testosterone levels.

Overall, rates of depression and anxiety are higher in PCOS samples, however, associations between BMI and mood and/or anxiety in PCOS samples are more complex. A systematic review
and meta-analysis found an increased risk for low mood in females with PCOS compared with healthy females, independent of BMI (Dokras et al., 2011). This finding is consistent with later studies which did not report significant associations between BMI and mood in PCOS samples (Blay et al., 2016; Dokras, 2012). Furthermore, one study found mood and anxiety symptoms to be similar in all phenotypes of PCOS, including obese females with infertility and hirsutism, who showed similar levels of depression compared with lean and fertile females (Veltman-Verhulst et al., 2012). Although two systematic reviews found higher mean values of BMI, insulin resistance, and hirsutism in women with PCOS and depression and anxiety compared with healthy controls, there were considerable methodological issues in these studies including strict inclusion criteria (further discussed in Chapter Five, section 5.3.1.2).

4.9 POLYCYSTIC OVARIAN SYNDROME AND COGNITIVE FUNCTION

4.9.1 Background

### Table 4.3

**Meta-Analytical Findings Related to Depression and Anxiety in Females with Polycystic Ovarian Syndrome**

<table>
<thead>
<tr>
<th>Study</th>
<th>Main goal</th>
<th>Number of participants</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barry et al. (2011)</td>
<td>Systematic review and meta-analysis (12 studies) examining symptoms of depression and anxiety in PCOS</td>
<td>12 studies assessing depression: 910-PCOS, 1347-HC; 6 studies assessing anxiety: 208-PCOS, 169-HC.</td>
<td>Significantly higher levels of mild depression and anxiety in PCOS patients compared with healthy controls. Lower BMI was found to be associated with lower levels of depression and anxiety.</td>
</tr>
<tr>
<td>Dokras et al. (2011)</td>
<td>Systematic review (17 studies) and meta-analysis (10 studies) examining symptoms of depression in PCOS</td>
<td>PCOS-522, HC-475</td>
<td>Four-fold increased risk for higher depression scores in PCOS females compared with healthy controls, independent of BMI or depression rating scales used.</td>
</tr>
<tr>
<td>Dokras et al. (2011)</td>
<td>Systematic review (9 studies) and meta-analysis (4 studies) examining symptoms of anxiety in PCOS</td>
<td>PCOS-450, HC-377</td>
<td>Significantly higher prevalence of generalised anxiety symptoms in females with PCOS compared with healthy controls. Increased risk for symptoms of anxiety in females with PCOS. Anxiety disorders including social anxiety disorder, panic attacks and obsessive compulsive disorder assessed less frequently.</td>
</tr>
<tr>
<td>Study</td>
<td>Main goal</td>
<td>Number of participants</td>
<td>Findings</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Veltman-Verhulst et al. (2012)</td>
<td>Meta-analytical study including 28 studies examining depression and 17 studies examining anxiety in females with PCOS</td>
<td>PCOS-2834</td>
<td>Majority of studies found higher levels of depression and anxiety in females with PCOS compared with healthy controls. Clinical hyperandrogenism did not account for emotional distress (QoL) in females with PCOS. BMI found to be unrelated to symptoms of depression in PCOS.</td>
</tr>
<tr>
<td>Blay et al. (2016)</td>
<td>Meta-analysis (six studies) including 5 studies examining symptoms of anxiety in PCOS and six examining symptoms of depression in PCOS</td>
<td>PCOS-343</td>
<td>Significantly higher anxiety and depression levels in females with PCOS compared with healthy controls.</td>
</tr>
<tr>
<td>Cooney et al. (2017)</td>
<td>Systematic review (30 studies) and meta-analysis (18 studies assessing depression and 9 assessing anxiety) in PCOS</td>
<td>PCOS-3050</td>
<td>Significantly increased odds of any symptoms of depression and anxiety and of moderate/severe symptoms of depression and anxiety in females with PCOS compared with healthy controls, independent of BMI. Meta-regression analysis findings included higher mean values of age, BMI, hirsutism in PCOS females with depression but no relationship between mood and testosterone levels. Females with PCOS and concurrent anxiety showed higher mean BMI values, hirsutism score and Free Testosterone levels compared with the control group.</td>
</tr>
</tbody>
</table>

PCOS- Polycystic Ovarian Syndrome, HC- Healthy Controls, BMI- Body Mass Index, QoL- Quality of Index
A unique opportunity lies in investigating the effect of testosterone on cognitive functioning in females with PCOS with high levels of testosterone, and to study differences in cognitive function before and after a pharmacological intervention. However, only a few studies until now have examined this relationship (Barnard, Balen, Ferriday, Tiplady, & Dye, 2007; Barry, Parekh, & Hardiman, 2013; Schattmann & Sherwin, 2007a, 2007b; Soleman et al., 2016). These studies will be discussed in the next section.

4.9.2 Cognitive function in PCOS

Cognitive function in females with PCOS is not an entirely well-studied area of research and of the few studies that have assessed cognitive function in females with PCOS, results are mixed. Overall, two studies suggested better spatial ability in females with PCOS (Barnard, Balen, et al., 2007; Barry et al., 2013) and two other studies suggested better executive functioning in females with PCOS following anti-androgen treatment (Schattmann & Sherwin, 2007a; Soleman et al., 2016).

Barry et al. (2013) examined visuospatial ability (Mental Rotation Test [MRT]) in females with PCOS \( n = 69 \) and found significantly better spatial ability in this sample compared with healthy controls \( n = 41 \). Additionally, a significant positive correlation was found between Free Testosterone levels and spatial ability within the PCOS group in this study, however, the same result was not found in the control group, which could be due to a small sample size of the study. Similarly, Barnard et al. (2007) found better performance (improved reaction time) on the spatial task in the PCOS group compared with the control group. In this internet-based study, 135 right-handed females with PCOS and healthy control females \( n = 322 \) were stratified based on anti-androgen treatment and the presence of symptoms of depression (Zung Depression Rating Scale) (Zung, 1965). The PCOS group using anti-androgen medication \( n = 69-109 \) was found to have significantly faster reaction time on the spatial task compared with
untreated PCOS group \((n = 66-110)\) suggesting that anti-androgen treatment may help improve reaction time on tasks assessing spatial ability. However, in this study, females with PCOS showed impaired performance in speed and accuracy on word recognition tasks (verbal learning and memory; considered to show a significant female-advantage). A similar impairment in performance on female-favouring tasks including verbal learning and memory, executive functions (verbal fluency, working memory) and psychomotor speed was observed in females with PCOS and hyperandrogenism \((n = 29)\) compared with healthy females \((n = 22)\) (Schattmann & Sherwin, 2007b). Compensatory performance on male-favouring cognitive tasks was not found in this study, which was, however, hampered by very low numbers. Another small randomised placebo-controlled trial investigated cognitive function in 19 females with PCOS before and after three months of anti-androgen (CPA) treatment (Schattmann & Sherwin, 2007a) and found no significant change in visuospatial ability and perceptual speed following treatment. Executive function (verbal fluency; COWAT) was found to improve post-treatment. A more recent small interventional study involving anti-androgen treatment examined working memory (executive functions) in females with PCOS using functional magnetic resonance imaging (fMRI) measures (Soleman et al., 2016). Baseline results showed greater levels of activation in the right superior and inferior parietal lobe during the memory task in the PCOS group \((n = 14)\) compared with the healthy control group \((n = 20)\). No significant difference was found in working memory scores (N-back Task involving letters) (Kane, Conway, Miura, & Colflesh, 2007) between the two groups. Post-treatment fMRI results showed no difference in brain activity between groups, suggesting a normalisation by treatment, along with increased accuracy in one aspect of the working memory task in females with PCOS.

Emotion processing has not been well-studied in females with PCOS. Only one neuroimaging study until now has examined emotion processing in females with PCOS (Marsh et al., 2013). In this small interventional study, females with insulin-resistant PCOS \((n = 7)\) were found to have greater limbic activation during the emotion processing task as found by fMRI, compared
with healthy controls \((n = 5)\), which reduced with treatment. Limbic areas including the prefrontal cortex, anterior cingulate, amygdala, and ventral basal ganglia/nucleus accumbens (known to be involved in the integration and evaluation of emotional information), have been implicated in depression. However, differences between groups in performance on tasks assessing emotion processing were unreported in this study.

In summary, one study showed significant cognitive impairment in females with PCOS, two studies found better spatial ability in females with PCOS, while two remaining studies found better executive functioning in females with PCOS following anti-androgen treatment. Further investigation is required to examine cognitive functioning in females with PCOS and high androgen levels.

### 4.10 CONCLUSION AND IMPLICATIONS

The underlying reason for the association between PCOS, mood and anxiety disorders still remains unclear (Cooney & Dokras, 2017). Neurobiological theories have been used to explain increased mood and anxiety symptoms in studies (Belmaker & Agam, 2008). Methodological issues such as selection of studies (exclusion of studies with incomplete reports), recruitment of participants from varying clinical settings, non-standardised tools and self-report measures used to assess symptoms, influence of covariates such as BMI, obesity and medications involved, lack of sensitivity analyses, and not accounting for heterogeneity of the syndrome have all led to contrasting clinical recommendations and an incomplete understanding of the aetiology of mood and anxiety disorders in PCOS (Blay et al., 2016; Dokras et al., 2011; Veltman-Verhulst et al., 2012).

It was recently documented that although the majority of physicians (>85%) were aware of cardio-metabolic comorbidities, very few were well-informed about the psychiatric distress
(depression and anxiety) involved in the syndrome (Dokras et al., 2017). Given the consistent evidence supporting the high prevalence of depression and anxiety in females with PCOS, it is imperative to implement appropriate treatment strategies that address the psychological issues involved. Polycystic Ovarian Syndrome has a significantly negative impact on health-related QoL, however, there is still a gap in both research and clinical practice related to causal factors behind mood and anxiety disorders in PCOS. The association between the biochemical aspects of the syndrome and psychiatric comorbidities need to be elucidated, particularly the association between the principal hormonal abnormality characteristic of PCOS: androgen excess, which may be related to mood, and therefore, low mood in PCOS could be resolved by normalising androgen levels. It has taken considerable time for guidelines by health professionals to recommend routine screening for mood symptoms in patients with PCOS (Center for Research Excellence in PCOS, 2015). Polycystic Ovarian Syndrome is not only a personally challenging and distressing experience for females who suffer from it but also a diagnostic challenge for clinicians. Therefore, it is essential to effectively treat the hormonal and psychological aspects of PCOS in a holistic manner, by promoting a healthy collaboration between endocrinologists and psychiatrists.
CHAPTER 5

ANDROGENS, DEPRESSION, ANXIETY, COGNITIVE FUNCTION AND EMOTION PROCESSING IN WOMEN:
A SYSTEMATIC REVIEW

5.1 INTRODUCTION

This chapter provides a systematic review of both cross-sectional and interventional studies that examined the association between androgen levels and mood, anxiety, cognitive function and emotion processing in females of reproductive age with and without PCOS.

5.1.1 Background

Depression is a serious and debilitating condition (see Chapter Two) and affects twice as many females as males in adulthood (Karger, 2014; Kessler, 2003; Kessler et al., 1994; Noble, 2005; Piccinelli & Wilkinson, 2000; Weissman et al., 1993; Weissman & Olfson, 1995). This difference in prevalence between genders, as well as increased prevalence of mood disorders in medical conditions associated with abnormal sex hormones, suggests that for at least some females, depression, and perhaps associated cognitive impairment, may have hormonal underpinnings. An example of an endocrine condition associated with an increased prevalence of mood disorders is PCOS. Polycystic Ovarian Syndrome is the most common endocrinological disorder in females of reproductive age (Azziz, Woods, et al., 2004; Bozdag et al., 2016; Farrell & Antoni, 2010; Franks, 1995; McGowan, 2011), affecting up to 5 to 22% of females, depending upon the diagnostic criteria (Azziz et al., 2016; Bozdag et al., 2016; Farquhar et al., 1994; Goodarzi & Azziz, 2006; Knochenhauer et al., 1998; Lizneva, Suturina,
et al., 2016; March et al., 2010; Mueller et al., 2014; Nestler, 1998; Sirmans & Pate, 2014; Teede et al., 2010). The high prevalence of depression and anxiety in females with PCOS is well-documented and associations between symptoms of depression and androgen excess have been indirectly implicated from prevalence and intervention studies in PCOS samples compared with healthy, age-matched females (Barry, Kuczmierczyk, et al., 2011; Bishop, Basch, & Futterweit, 2009; Blay et al., 2016; Cooney & Dokras, 2017; Cooney et al., 2017; Deeks et al., 2010; Dokras, 2012; Dokras et al., 2011, 2012; Mansson et al., 2008; Veltman-Verhulst et al., 2012) (see Chapter Four). However, there is limited understanding of the direct effect of androgens on the female brain and the clinical manifestations of this. Additionally, a slightly increased rate of depression is found in patients with PCOS and large numbers of other women who are treated with the OCP and similar hormonal agents aiming to reduce androgen levels (Skovlund, Morch, Kessing, & Lidegaard, 2016). It is important to consider whether the two are linked and to consider other possible cognitive/emotional aspects of lowered androgen levels.

Data on testosterone levels in depression and during treatment is equivocal. Significant increases in testosterone levels in females have been reported following antidepressant treatment (Cohen, 1999; Giltay et al., 2012; Kumsar et al., 2014). However, these findings have not been consistently replicated, with other studies, including one longitudinal study, reporting significantly higher blood concentrations of Total Testosterone at baseline in untreated depressed females compared with healthy control females (Baischer et al., 1995) and decreased Free Testosterone levels in patients (12 males and eight females) treated with antidepressants compared with the placebo condition in a double-blind placebo controlled study (Cohen, 1999). These findings, and those in PCOS, suggest that testosterone (and perhaps other androgens) may play a role in the development of mood disorders for a subset of females. However, the exact nature of this relationship is unclear due to lack of direct measurement of androgen levels in relation to mood symptoms in PCOS samples.
There is a suggestion of worse cognitive function in the areas of memory, attention, and executive function in females with PCOS compared with healthy females (Barnard, Balen, et al., 2007; Schattmann & Sherwin, 2007b). Whether this worse performance is related to symptoms of depression or is an independent feature of androgen excess has not been examined yet. The finding of gender differences in cognitive function, with males generally outperforming females on tests of mathematical and visuospatial ability (Benbow, 1988; Driscoll et al., 2005; D. Goldstein et al., 1990; L. Harris, 1981; Linn & Petersen, 1985; N. Watson & Kimura, 1991; Wittig & Petersen, 1979) and females outperforming males in tests of verbal memory, processing speed, and verbal fluency (Halpern, 2000) suggests an organisational effect of sex hormones (previously discussed in Chapter Three, section 3.4.2) and does not necessarily imply an effect of ambient androgen levels since, of course, other hormones vary between genders. Research suggests that females with PCOS, however, tend to show worse performance on tasks of verbal and spatial memory, processing speed and attention and executive function (verbal fluency) compared with healthy females (Barnard, Balen, et al., 2007; Schattmann & Sherwin, 2007b). Findings from neurobiological studies indicate a wide distribution of androgen receptors in brain regions fundamental for learning and memory, particularly the hippocampus (Beyenburg et al., 2000; Brännvall, Bogdanovic, Korhonen, & Lindholm, 2005; Hamson et al., 2013; Xiao & Jordan, 2002), temporal cortex (Puy et al., 1995; Sarrieau et al., 1990), and the prefrontal cortex, which is not prominently involved in learning and memory function but plays a role (Finley & Kritzer, 1999). However, recent studies have found that there may be other factors including epigenetic factors such as psychological, socio-cultural and environmental that may affect sex differences in cognitive function, which warrant further investigation (Levine, Foley, Lourenco, Ehrlich, & Ratliff, 2016; D. Miller & Halpern, 2014).

Emotion processing is the ability to detect and decode facial emotional expressions and is required for successful interpersonal relationships. Research suggests significant emotion-specific deficits in MDD (Bourke et al., 2010). Reviews have suggested that individuals with
depression show abnormal facial emotion processing observed in the form of impaired recognition accuracy, misinterpretation of neutral faces as sad, and attentional biases to sad faces (Bouhuys et al., 1999; Bourke et al., 2010; Stuhrmann et al., 2011). The amygdala, which is involved in processing emotional material (Kesler et al., 2001; Morris et al., 1998), has been well-documented to mediate gender differences in emotion processing (Hamann, 2005; Kret & De Gelder, 2012; Schienle, Schäfer, Stark, Walter, & Vaitl, 2005; S. Schneider et al., 2011), has a high density of androgen receptors (Abdelgadir, Roselli, Choate, & Resko, 1999; Sarrieau et al., 1990; Simerly et al., 1990; Zuloaga, Puts, Jordan, & Breedlove, 2008b), and its activity has been found to be influenced by sex hormones including testosterone (van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010).

Despite these indicative findings, relatively limited research has directly assessed the relationship between androgen abnormalities and symptoms of depression and anxiety in reproductive-aged females. Even less is known of the direct association between androgen levels and cognition and emotion processing. Importantly, knowledge of this association will help inform endocrinologists about the complexities of PCOS from a mental health perspective. For this purpose, existing literature was reviewed on the direct relationship between abnormalities in the androgen system in females and the psychological symptoms of depression and anxiety, together with associated features of brain function including cognitive function, and emotion processing.

5.1.2 Aims

This review aims to examine the role played by androgen excess in mood and anxiety, and related worse cognitive performance, in reproductive-aged females with and without PCOS. The current review was inclusive in sampling, in that it was inclusive of both cross-sectional and interventional studies relating mood, anxiety and cognitive function in reproductive-aged female samples with PCOS and without PCOS.
This review has two overarching aims: 1) to provide a clear understanding of the relationship between androgens, mood, anxiety, cognitive function and emotion processing in females of reproductive age, and 2) to identify methodological issues of research in this area.

5.2 METHOD

5.2.1 Search Strategy

Electronic database searches were carried out for relevant papers using PubMed, Google Scholar and Web of Science up to January 2017. In the initial search, the following terms were used:

- ‘androgen’ or ‘testosterone’ or ‘polycystic ovary syndrome’ or ‘polycystic ovarian disorder’, and
- ‘major depressive disorder’ or ‘depression’, or ‘anxiety’, and
- ‘cognitive function’ or ‘neuropsychological function’, or ‘spatial ability’ or ‘emotion processing’ or ‘facial expression recognition’.

Reference lists for all relevant papers were checked and Web of Science was used to review the articles that had cited relevant articles found using these search strategies.

5.2.2 Inclusion criteria

Inclusion criteria were as follows:

- Interventional or observational studies that involved an assessment of symptoms of depression/anxiety and/or cognitive function and/or emotion processing AND androgen levels,
- Non-pregnant, pre-menopausal females aged between 16 and 45 years (PCOS or non-PCOS,
- English-language publications.
5.2.3 Exclusion criteria

Studies were excluded for the following reasons: (i) inclusion of male and female participants without a separate analysis of results for the female sample, (ii) androgen levels not reported (often because androgen levels were used as a screening tool in PCOS studies), (iii) lacking analyses directly investigating associations between androgen levels and mood/cognitive function/emotion processing, and (iv) use of a non-standardised measure of mood or anxiety symptoms.

5.3 RESULTS AND DISCUSSION

A total of 73 studies met the inclusion criteria, by including a direct examination of the relationship between androgens and mood/anxiety/cognitive function/emotion processing in reproductive-aged females (see Tables 5.1, 5.2, 5.3, 5.4). Thirty-three studies specifically examined associations between androgen levels and symptoms of depression and anxiety symptoms, of which 21 studies included PCOS samples (see Table 5.1) and 12 included non-PCOS samples (see Table 5.2). Of the remaining forty studies, 21 used an observational study design (see Table 5.3) and 19 used an experimental/interventional study design (see Table 5.4) to examine the relationship between androgen levels and cognitive function and emotion processing.

Measures of Androgens

A range of androgens were analysed including: Testosterone (Total Testosterone, Free Testosterone, generally used synonymously with Bioactive and Bioavailable Testosterone), Dihydrotestosterone (DHT), Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone Sulfate (DHEA-S), Androstenedione (A-dione), Androstenediol (A-diol) and Free Androgen Index (FAI) (ratio of Total Testosterone to SHBG multiplied by a 100). Sex Hormone Binding Globulin (SHBG) was also assessed by several studies.
Free Testosterone appears to be a more useful measurement than Total Testosterone since only Free Testosterone is available to act at the receptors (Huang et al., 2010) (see Chapter 3, Section 3.3.1). Therefore, studies investigating the relationship between Free Testosterone/FAI and psychological measures are likely to be more relevant than those which examine Total Testosterone levels (Vermeulen et al., 1999). Other androgens are similar to testosterone in their action, with the exception of DHEA, DHEA-S and A-dione, which are pro-androgens or precursors to testosterone (see Chapter Three). Given its somewhat different profile, it is worth considering the effects of DHEA-S quite separately from those of other androgens. However, the best androgen measures for the purpose of this review remains to be Free Testosterone or FAI.

Another important methodological issue that was identified was a consideration of the variability of testosterone levels or the point in the range of testosterone levels at which a group is functioning. Some studies included PCOS patients (higher than normal testosterone levels) while others included anorexia nervosa and HIV patients (lower androgen levels) and transsexuals undergoing cross-sex hormone treatment (with varied androgen levels). Additionally, there are many other issues related to testosterone assessment or administration including diurnal/seasonal fluctuations and fluctuations related to menstrual phases. First, one study suggested that 24-hour mean plasma testosterone concentrations showed a steep decline at age 40 (age range included 21-51 years) in non-obese females with regular menstrual cycles (Zumoff et al., 1995). Additionally, testosterone levels in females are subject to small but significant fluctuations in testosterone levels during different menstrual phases (Goebelmann, Arce, Thornycroft, & Mishell, 1974; Judd & Yen, 1973). Goebelmann et al. (1974) found that Total Testosterone levels were highest around midcycle LH peak and higher during the follicular phase compared with the luteal phase of the cycle. Additionally, averages for individual participants varied significantly for the entire cycle. However, the study concluded that mean testosterone levels largely fell into a relatively narrow range. Free Testosterone levels were not assessed in the Goebelmann et al. study. Although it has been suggested that sexually dimorphic cognitive skills
that show a significant male-advantage improve during menstrual phases involving low oestrogen, and cognitive tasks with a female-advantage improve in association with high oestrogen levels (Hampson, 1990; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Güntürkün, 2000; Nyborg, 1983; S. Phillips & Sherwin, 1992), a recent review and meta-analysis did not find robust evidence related to the influence of menstrual cycle on cognitive function (Poromaa & Gingnell, 2014). This review, however, suggested an association between improvement in performance on tasks assessing verbal or spatial working memory and higher levels of oestradiol. There was limited evidence for this finding, however, and is not the main topic of interest for the current review. Additionally, in Poromaa et al’s study (2014), emotion-processing related changes were more consistently found to be associated with progesterone, however, no significant findings related to testosterone levels were reported.

Issues regarding testosterone administration include the type of administration, i.e., gel, patch or injection, and the determination of the most effective time period between exogenous administration and testing (Gordon, Corbin, & Lee, 1986; Postma et al., 2000). This may help clarify results, since in a small cross-sectional study involving collection of multiple samples of testosterone, van Honk et al. (1999) found a significant association between emotion processing of angry faces (attending away from threatening faces), mood (Profile of Mood States: POMS) and Free Testosterone samples collected six hours prior to testing (showing highest Free Testosterone levels of all samples taken), but not with testosterone samples taken at other time-points in 16 healthy females. However, this may likely be a chance finding due to multiple samples collected prior to the emotion processing assessment. Regarding interventional studies involving testosterone administration, studies have repeatedly shown that there is a tenfold increase in Total Testosterone following testosterone administration returning to baseline within 90 minutes, with no changes in SHBG. However, Free Testosterone levels have not been reported to increase in these studies (Bos et al., 2013; Hermans et al., 2007; Postma et al., 2000; Schutter & van Honk, 2004; Tuiten et al., 2000; van Honk, Peper, & Schutter, 2005; van Honk et al., 2001).
### Table 5.1

**Studies Examining the Relationship between Androgen Levels, Mood and Anxiety in Females with Polycystic Ovarian Syndrome (21 studies)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Study design and key measures</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Rocco et al. (1991) | 21 Polycystic Ovarian Syndrome females (PCOS), 10 healthy controls (HC) | PCOS = 20.2, HC = 21.7 | Cross-sectional study design  
Clinical measures: Minnesota Multiphasic Personality Inventory (MMPI), State-Trait Anxiety Inventory (STAI).  
Androgen measures: Total testosterone (Total T), Androstenedione (A-dione), Dehydroepiandrosterone sulphate (DHEA-S). | Depressed and non-depressed PCOS groups identified based on MMPI scores (>upper limit of 70) and STAI (>upper limit of 44, 48).  
Higher A-dione levels in depressed females with PCOS (n = 11) compared with non-depressed females with PCOS (n = 10).  
No significant correlation between androgen levels and anxiety. |
| Ragson et al. (2003) | 32 PCOS                               | Unstated*          | Cross-sectional study design  
Clinical measures: Centre for Epidemiological Studies-Depression Rating Scale (CES-D).  
Androgen measures: Bioavailable T (Free Testosterone: Free T). | No significant correlation between depression (CES-D) and Free T levels. |
| Weiner et al. (2004) | 27 PCOS and 27 HC                     | PCOS = 28.2, HC = 30 | Cross-sectional study design  
Clinical measures: Visual Analogue Scale (VAS: 6 negative and 4 positive moods), STAI, State-Trait Depression Adjective Check List.  
Androgen measures: Total T, Free T. | Significant positive correlation between Free T levels and anxiety (STAI) in HC group.  
Significant negative correlation between state anxiety and Free T levels in PCOS group.  
No significant relationship between mood and Free T levels in PCOS group.  
Curvilinear relationship between Free T levels and mood and anxiety with highest depression and anxiety levels at mid-range (10-26 pg/ml) of Free T levels (analysis included both groups) |
| Hahn et al. (2005)  | 120 PCOS and 50 HC                    | PCOS = 29, HC = 30 | Cross-sectional study design  
Clinical measures: Symptom Checklist Survey 90 R (SCL-90-R) to measure of mood and anxiety.  
Androgen measures: Total T | No significant correlation between Total T levels and mood or anxiety. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Study design and key measures</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Hollinrake et al. (2007)     | 103 PCOS and 103 HC                 | PCOS = 29.8    | *Cross-sectional study design*  
  *Clinical measures:* Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PRIME-MD PHQ) to measure anxiety and mood and Beck Depression Inventory (BDI) to measure depression.  
  *Androgen measures:* Total T, Free T, DHEA-S. | No significant difference in androgen levels between depressed ($n = 36$) and non-depressed ($n = 67$) PCOS females.  
  No significant correlation between depression (BDI) and androgen levels or between anxiety and androgen levels. |
| Soyupek et al. (2008)        | 37 PCOS and 35 HC                   | PCOS=24.10±6.13| *Cross-sectional study design*  
  *Clinical measures:* BDI  
  *Cognitive measures:* Psychomotor speed (Manual dexterity) measured by the Grooved Pegboard Test  
  *Androgen measures:* Total T, DHEA-S. | No significant correlations between mood and androgen levels.  
  No significant correlations between manual dexterity and androgen levels. |
| Adali et al. (2008)          | 42 PCOS and 42 HC                   | PCOS=23.5      | *Cross-sectional study design*  
  *Clinical measures:* General Health Questionnaire (GHQ-12) to measure psychiatric distress, BDI  
  *Androgen measures:* Total T, DHEA-S | No significant correlation between mood (BDI) and androgen levels. |
| Mansson et al. (2008)        | 49 PCOS and 49 HC                   | Both groups-35.9| *Cross-sectional study design*  
  *Clinical measures:* Mini International Neuropsychiatric Interview (MINI).  
  *Androgen measures:* Total T, Free Androgen Index (FAI) (to estimate Free T). | Total T and Free T levels not significantly correlated with Major Depressive Disorder (MDD) (including history of MDD) in PCOS group.  
  FAI levels higher in PCOS group with a lifetime incidence of social anxiety disorder compared with PCOS females without anxiety. |
| Bhattacharya & Jha (2010)    | 117 PCOS (75 with depression, 42 without depression) and 84 HC | PCOS with depression-21.9  
  Without depression- 21.1  
  HC = 21-32 | *Cross-sectional study design*  
  *Clinical measures:* PRIME-MD PHQ-9.  
  *Androgen measures:* Total T. | No significant difference in Total T levels between PCOS females with ($n = 75$) and without ($n = 42$) depression. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Study design and key measures</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Jedel et al (2011)           | 72 PCOS females: 35 depressed and 37 non-depressed | 28.5           | Cross-sectional study design  
Clinical measures: Microneurography to measure sympathetic nerve activity (to measure affective symptoms), Comprehensive Psychopathological Rating Scale For Affective Syndrome to measure depression and anxiety, Montgomery-Asberg Depression Rating Scale (MADRS)-self rated for assessing mood and Brief Scale for Anxiety (BSA-A)  
Androgen measures: Total T, Free T, Dehydroepiandrosterone (DHEA), DHEA-S, A-dione, Androstenediol (A-Diol), Dihydrotestosterone (DHT). | Lower Total T and Free T levels (and glucuronidated androgen metabolite -3G) (< 10 pg/ml) in PCOS females with higher scores on depression rating scale (MADRS-S>11) (n = 35) compared with PCOS group with lower depression scale scores (n = 37).  
Binary logistic regression analyses showed significant negative correlation between Free T levels and depression (lower Free T and 3G levels associated with higher scores on MADRS-S>11)  
No significant association between androgen levels and anxiety. |
| Barry et al (2011)           | 76 PCOS and 49 subfertile HC       | PCOS = 28.8 HC = 35.1 | Cross-sectional study design  
Clinical measures: Hospital Anxiety and Depression Scale (HADS), and Polycystic Ovarian Syndrome Questionnaire (PCOS-Q) for assessing hyperandrogenic symptoms.  
Androgen measures: Total T | No significant correlation between Total T levels and mood or anxiety in either group. |
| Livadas et al (2011)         | 130 females with PCOS (54 normal anxiety, 46 moderate anxiety 30 severe anxiety) | Normal = 25.2 Moderate = 25.9 Severe = 2437 | Cross-sectional study design  
Clinical measures: STAI (Trait and State), BDI.  
Androgen measures: Total T, Free T, A-dione, DHEA-S, FAI. | No significant correlation between mood (BDI) and androgen levels.  
Significant positive correlation between anxiety and FAI levels, higher STAI-S (not STAI-T) scores associated with higher FAI levels (significance value p < 0.05 between patients with normal and moderate anxiety). |
| Pastore et al (2011)         | 94 PCOS and 96 HC                 | PCOS= 27.2 HC= 30-34 | Cross-sectional study design  
Clinical measures: Quick Inventory of Depressive Symptomatology Self-Report (QIDS)  
Androgen measures: Total T, Free T, DHEA-S. | Androgen levels not significantly correlated with depression in PCOS females. |
| Cinar et al (2011)           | 226 PCOS 85 HC                    | PCOS= 23.3 HC= 24.4 | Cross-sectional study design  
Clinical measures: BDI, STAI, HADS, General Health Questionnaire (GHQ), PCOS-Health Related Quality of Life (PCOS- HRQoL) (to assess emotions, hirsutism, weight, infertility and menstrual problems)  
Androgen measures: Total T, FAI. | Significant positive correlation between FAI levels and mood (HADS not BDI) (higher FAI levels associated with higher score on depression sub-scale of HADS).  
Significant positive correlation between FAI levels and state anxiety (higher FAI levels associated with higher scores on STAI-S (not STAI-T). |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Study design and key measures</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moran et al. (2012)</td>
<td>54 PCOS females (National Institutes of Health (NIH) Criteria) N = 29 and non-NIH N = 25 groups) and 27 HC</td>
<td>NIH PCOS=32 Non-NIH PCOS=33.4 HC = 36.4</td>
<td>Cross-sectional study design Clinical measures: Polycystic Ovarian Syndrome Questionnaire Health Related Quality of Life (PCOS-QHRQoL) and HADS Androgen measures: Total T, FAI.</td>
<td>Multivariate regression analyses showed FAI levels and age to significantly predict depression. No association between androgen levels and anxiety.</td>
</tr>
<tr>
<td>Annagür et al. (2013)</td>
<td>Total n = 73 PCOS with: MDD (23), PCOS with Generalized Anxiety Disorder (GAD)(20) and PCOS with no psychiatric Diagnosis (ND)(30)</td>
<td>MDD = 21.7 GAD = 22.2 ND = 21.9</td>
<td>Cross-sectional study design Clinical measures: Structured Clinical Interview for DSM-IV (SCID-I) Androgen measures: Free T, DHEA-S.</td>
<td>DHEA-S levels significantly higher in PCOS females with MDD and PCOS females with GAD group (no difference in androgen levels between these two psychiatric groups) compared with PCOS group with no psychiatric diagnosis.</td>
</tr>
<tr>
<td>Milsom et al. (2013)</td>
<td>102 PCOS and 1349 HC</td>
<td>PCOS = 17 HC = 14-19</td>
<td>Cross-sectional study design (survey) Clinical measures: Reynolds Adolescent Depression Scale (RADS-2) Androgen measures: Total T</td>
<td>No significant correlation between Total T levels and depression (RADS-2 scores) in both groups.</td>
</tr>
<tr>
<td>Bazarganipour et al. (2013)</td>
<td>300 PCOS</td>
<td>26.56 yrs</td>
<td>Cross-sectional study design Clinical measures: HADS Androgen measures: Total T, FAI.</td>
<td>Significantly higher FAI levels in depressed and anxious females with PCOS compared with PCOS females without symptoms of depression or anxiety.</td>
</tr>
<tr>
<td>Rahiminejad et al. (2014)</td>
<td>120 PCOS</td>
<td>Mean age- 24</td>
<td>Cross-sectional study design Clinical measures: BDI Androgen measures: Total T</td>
<td>No significant difference in Total T levels between depressed and non-depressed females with PCOS. No significant correlation between BDI and Total T levels.</td>
</tr>
<tr>
<td>Klimczak et al. (2015)</td>
<td>60 PCOS divided into groups based on age (&lt;27 years) and depression scores (PHQ9 and QIDS)</td>
<td>(age-range 19-46) Median age- 27</td>
<td>Cross-sectional study design Clinical measures: BDI, QIDS, PHQ9 Androgen measures: DHEA-S, Total T, A-dione, FAI.</td>
<td>No significant differences in androgen levels between depressed and non-depressed groups (QIDS and PHQ).</td>
</tr>
<tr>
<td>Asik et al. (2015)</td>
<td>71 PCOS, 50 HC</td>
<td>PCOS=22 HC=24</td>
<td>Cross-sectional study design Clinical measures: HADS Androgen measures: Total T, DHEA-S</td>
<td>No significant correlations between mood and androgen levels in the entire sample</td>
</tr>
</tbody>
</table>
Table 5.1 Continued

ABS- Affect Balance Scale, Androstenedione, Androstenediol, A-dione- Androstenedione, A-diol- Androstenediol, BDI- Beck Depression Inventory, Bioactive T = Biologically Active Testosterone, BMI = Body Mass Index, BSA- Brief Scale for Anxiety, CES-D- Centre for Epidemiological Studies- Depression Rating Scale, COWAT- Controlled Oral Word Association Test, DHEA= Dehydroepiandrosterone, DHEA-S= Dehydroepiandrosterone Sulfate, DSI- DeRogatis Symptom Inventory, FAI- Free Androgen Index, Free T- Free Testosterone, GHQ-12- General Health Questionnaire, HC = healthy controls, HADS- Hospital Anxiety and Depression Scale, HAM-A- Hamilton Rating scale for Anxiety, HAM-D- Hamilton Depression Rating Scale, ICD-10- International Classification of Diseases, MAACL- Multiple Affect Adjective Check List, MADRS- Montgomery-Asberg Depression Rating Scale, MINI- Mini International Neuropsychiatric Interview, MMPI- Minnesota Multiphasic Personality Inventory, PRIME-MD PHQ- Primary Care Evaluation of Mental Disorders Patient Health Questionnaire, PCOS-Q- Polycystic Ovarian Syndrome Questionnaire, POMS- Profile Of Mood States, PCOS = Polycystic Ovarian Syndrome, PL = placebo group, QIDS- Quick Inventory of Depressive Symptomatology, SCL-90-R- Symptom Checklist Survey 90 R, SCID-I- Structured Clinical Interview for DSM-IV, STAI- State-Trait Anxiety Inventory, Total T = Total Testosterone, VAS- Visual Analogue Scale, ZARS- Zung Anxiety Rating Scale.
### Table 5.2

**Studies Examining the Relationship between Androgen Levels, Mood and Anxiety in Reproductive-aged Females without Polycystic Ovarian Syndrome (12 studies)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Mean age (yrs)</th>
<th>Measure of Interest</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Shulman et al    | 20 females with facial hirsutism            | 27.5           | Cross-sectional study design
Clinical measures: DeRogatis Symptom Inventory (DSI) for general mental health symptoms and Affect Balance Scale (ABS) (with 4 negative and 4 positive mood states)
Androgen measures: Total T, Free T, Bioactive Testosterone (Bioactive T), DHEA, DHEA-S, A-dione. | Significant positive correlation between depression (DSI score) and Free T levels (higher Free T levels associated with higher depression scores). Significant correlations between Bioactive T levels and depression (ABS), with a negative correlation between Bioactive T and positive affect (ABS) and positive correlation between Bioactive T and negative affect. No significant association between androgens and anxiety levels. |
| De Rogatis et al.| 20 females with idiopathic hirsutism        | 27.5           | Cross-sectional study design
Clinical measures: DSI, ABS
Androgen measures: Total T, Free T, Bioactive T, DHEA, DHEA-S, A-dione. | Significant positive correlation between Free T and Bioactive T levels, depression (DSI and ABS) and phobic anxiety (DSI) (higher Free T levels associated with higher scores on depression rating scale and phobic anxiety sub-scale). Significant negative correlation between Free T and Bioactive T levels and total positive affect score (ABS). No significant association between Total T, DHEA, DHEA-S and A-dione levels and mood or anxiety. |
| Landen et al     | 216 females with (n = 31) and without (n = 185) social anxiety | Age range 41-42 | Cross-sectional study design
Clinical measures: DSM-IV based self-report questionnaire to measure social anxiety and related psychiatric conditions including depressed mood, anxiety attacks, bulimia nervosa, obsessive-compulsive disorder and premenstrual dysphoric disorder.
Androgen measures: Total T, Free T, DHEA-S. | Total T levels significantly lower in social anxiety group compared with HC. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Mean age (yrs)</th>
<th>Measure of Interest</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller et al.</td>
<td>43 Females with anorexia nervosa with and without depression and anxiety</td>
<td>26.3</td>
<td>Cross-sectional study design</td>
<td>Significant negative correlation between Total and Free T levels, and mood and anxiety severity in anorexic females, (lower levels of Total and Free T associated with higher anxiety (HAM-A&gt;18) and higher depression scores (HAM-D&gt;12) compared with anorexic females without depression and anxiety. Step-wise regression analysis showed Free T levels to significantly predict depression and anxiety severity.</td>
</tr>
<tr>
<td>Roepke et al</td>
<td>31 Borderline Personality Disorder (BPD) patients (PCOS-7, non PCOS-16) and 30 HC (PCOS-2, non PCOS-27)</td>
<td>BPD- 31 HC- 30</td>
<td>Cross-sectional study design</td>
<td>Multiple regression analyses showed a significant positive correlation between symptoms of low mood (HAM-D) and FAI levels in BPD group (higher scores on HAM-D associated with higher levels of FAI), not significant after controlling for BMI. Total T, FAI and A-dione levels significantly elevated in BPD group.</td>
</tr>
<tr>
<td>Zheleznova et al</td>
<td>101 females with epilepsy and anxiety and/or depression</td>
<td>29.43</td>
<td>Cross-sectional study design</td>
<td>Significant negative correlation between T levels and depression and anxiety (higher T levels associated with lower scores on depression scale (HADS and SCL-90) and anxiety (HADS) in epileptic patients with mild depressive disorder (n = 58).</td>
</tr>
<tr>
<td>Schutter et al</td>
<td>76 healthy females</td>
<td>17.4</td>
<td>Cross-sectional study design</td>
<td>No significant correlation between T levels and anxiety and depression in females.</td>
</tr>
<tr>
<td>Baischer et al</td>
<td>20 depressed females and 10 HC</td>
<td>32.5</td>
<td>Intervventional study (T levels measured before and during clomipramine treatment for 4 weeks)</td>
<td>At baseline, significantly elevated Total T levels in untreated depressed patients (HAM-D &gt;14) compared with HC. Antidepressant treatment followed by decreased T levels in depressed patients. No significant differences in T levels between HC group and patient group post clomipramine treatment.</td>
</tr>
<tr>
<td>Study</td>
<td>Sample</td>
<td>Mean age (yrs)</td>
<td>Measure of Interest</td>
<td>Key findings</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>----------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Wolkowitz et al (1999)</td>
<td>10 females with MDD (5 in DHEA group, 5 in placebo) (patients were either medication-free or stabilized on antidepressants)</td>
<td>44</td>
<td>Double-blind placebo-controlled study design involving 30 mg DHEA treatment for 8 weeks</td>
<td>DHEA group showed significantly greater decrease in depression (HAM-D scores) compared with placebo group.</td>
</tr>
<tr>
<td>Schutter et al (2005)</td>
<td>14 healthy females</td>
<td>21.6</td>
<td>Randomised double-blind placebo-controlled crossover study design involving 0.5 mg T administration (with two testing sessions separated by 48 hours)</td>
<td>No significant differences in self-reported mood or anxiety following T administration compared with placebo condition.</td>
</tr>
<tr>
<td>Dolan Looby et al (2009)</td>
<td>25 HIV-infected females (with Free T levels below normal range), 13 under treatment and 12 in placebo group</td>
<td>Treatment = 13 Placebo = 12</td>
<td>Randomised placebo-controlled trial (involving active transdermal T administration (500 mcg twice weekly) over 18 months)</td>
<td>Significant improvement in mood (BDI indices) following 18 months of T administration compared with placebo.</td>
</tr>
<tr>
<td>Kumsar et al (2014)</td>
<td>Females with (n = 52) and without (n = 30) major depression</td>
<td>Depressed group- 31.3 HC-33.1</td>
<td>Interventionsal study involving SSRI anti-depressant treatment (50 mg Sertraline) for six weeks.</td>
<td>Pre-treatment Total T and FAI levels significantly lower in depressed group compared with HC. Significant increases in Total T and FAI levels following antidepressant treatment. No significant difference in post-treatment androgen levels between the two groups.</td>
</tr>
</tbody>
</table>

ABS- Affect Balance Scale, A-dione-Androstenedione, A-diol- Androstenediol, BDI- Beck Depression Inventory, Bioactive T = Biologically Active Testosterone, BMI = Body Mass Index, BSA-Brief Scale for Anxiety, CES-D-Centre for Epidemiological Studies Depression Rating Scale, DHEA= Dehydroepiandrosterone, DHEA-S=Dehydroepiandrosterone Sulfate, DSM- DeRogatis Symptom Inventory, FAI- Free Androgen Index, Free T- Free Testosterone, GHQ-12-General Health Questionnaire, HC = healthy controls, HRM = hormone group, HADS- Hospital Anxiety and Depression Scale, HAM-A- Hamilton Rating scale for Anxiety, HAM-D- Hamilton Depression Rating Scale, ICD-10- International Classification of Diseases, MAACL- Multiple Affect Adjective Check List, MADRS- Montgomery-Asberg Depression Rating Scale, MINI- Mini International Neuropsychiatric Interview, MMPI- Minnesota Multiphasic Personality Inventory, PRIME-MD PHQ- Primary Care Evaluation of Mental Disorders Patient Health Questionnaire, PCS-Q- Polycystic Ovarian Syndrome Questionnaire, POMS- Profile Of Mood States, PCOS = Polycystic Ovarian Syndrome, PL = placebo group, QIDS- Quick Inventory of Depressive Symptomatology, SCL-90-R- Symptom Checklist Survey 90 R, SCID-I- Structured Clinical Interview for DSM-IV, STAI-State-Trait Anxiety Inventory, Total T = Total Testosterone, VAS- Visual Analogue Scale, ZARS- Zung Anxiety Rating Scale, *= Insufficient information about the study sample provided by the authors – contact with corresponding author of the study was attempted at least twice, with no response.  

102
Table 5.3
Cross-sectional Studies Examining the Relationship between Androgen Levels, Cognitive Function and Emotion Processing in Reproductive-aged Females with and without Polycystic Ovarian Syndrome (21 studies)

<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Measures of Interest</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shute et al (1983) (Study 1 and 2)</td>
<td>Study 1: 48 healthy females Study 2: 12 females with highest and lowest androgen levels ($n = 6$ each) (selected from a total sample of 33)</td>
<td>Study 1: 24.5 Study 2: Unstated</td>
<td>Study 1: Cross-sectional study design Cognitive measures: Visuospatial ability: Six timed-tests from the French Reference Kit for Cognitive Factors (including 2 tests each from 3 factors namely flexibility of closure, spatial orientation and visualization) Androgen measures: Free T Study 2: Cross-sectional study design Cognitive measures: Visuospatial ability: Minnesota Paper Form Board Test (MPFB), Primary Mental Abilities Test (PMA) and Comprehensive Ability Battery Space Test (CAB-S) to measure Mental Rotation. Androgen measures: Free T</td>
<td>Study 1: Regression analyses showed no significant correlation between Free T levels and spatial ability in females. Study 2: Significant difference between two groups in spatial performance with high-androgen group performing significantly better compared with low-androgen group on spatial visualisation (MFPB scores).</td>
</tr>
<tr>
<td>McKeever et al (1987)</td>
<td>19 left-handed and 23 right-handed healthy females</td>
<td>*Unspecified</td>
<td>Cross-sectional study design Cognitive measures: Visuospatial ability- Stafford Identical Blocks Test, MPFB Test. Verbal Ability- Shipley Hartford Vocabulary Test Executive Function-Verbal Fluency Test. Androgen measures: Total T</td>
<td>No significant correlation between spatial or verbal ability measures and T levels in both groups.</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean age (yrs)</td>
<td>Measures of Interest</td>
<td>Main Findings</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------------------------------------</td>
<td>----------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Gouchie and Kimura (1991)</td>
<td>46 females divided into high and low T groups.</td>
<td>21.5</td>
<td>Cross-sectional study design with two test sessions (only baseline scores used). Cognitive measures: Visuospatial ability- Paper Folding, Mental Rotations Test (MRT) (Vandenberg &amp; Kuse). Verbal ability- verbal articulation (Tongue-Twister Task) Psychomotor speed- Finding A’s Test and Identical Pictures Test (both from ETS kit of Factor-Referenced Cognitive Tests) Mathematical ability- Mathematics Aptitude Test General intelligence- Advanced Vocabulary Test Androgen measures: Free T</td>
<td>Significantly better visuospatial ability performance (Paper Folding scores) in high T group compared with low T group. Multiple regression analysis found no significant correlations between T levels and cognitive performance.</td>
</tr>
<tr>
<td>Hassler et al (1992)</td>
<td>25 healthy females</td>
<td>18.77</td>
<td>Cross-sectional study design. Cognitive measures: Visuospatial ability-Spatial Relations Test, Hidden Pattern Test, and Witelson’s Dichaptic Stimulation Test. Executive function- verbal fluency measured by German version of Primary Mental Abilities Test. Androgen measures: Total T</td>
<td>Total T levels not significantly correlated with cognitive function.</td>
</tr>
<tr>
<td>Phillips et al (1992)</td>
<td>25 healthy females</td>
<td>24</td>
<td>Repeated-measures study (females tested in menstrual and luteal phase in a counterbalanced manner). Clinical Measures: Multiple Affect Adjective Check List (MAACL) to measure anxiety and mood Cognitive tests: Verbal Memory- Wechsler Memory Scale including four sub-scales-Logical memory/Paragraph Recall, Visual Reproduction, Associate Learning Executive function- Digit Span. Androgen Measure: Free T.</td>
<td>Free T levels significantly negatively correlated with verbal memory (lower levels of Free T within luteal phase associated with better performance) in delayed recall of paragraphs. No significant correlation between mood (MAACL scores) and Free T levels.</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean age (yrs)</td>
<td>Measures of Interest</td>
<td>Main Findings</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------</td>
<td>----------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Moffat and Hampson (1996)</td>
<td>40 healthy females: Right-handed -19, Left-handed -21</td>
<td>23</td>
<td><strong>Cross-sectional study design</strong>&lt;br&gt;<strong>Cognitive measures:</strong>&lt;br&gt;Visuospatial ability: Paper Folding Test (PFT) and MRT Executive Function: verbal fluency measured by Controlled Associations Test and Controlled Oral Word Association Test (COWAT) Androgen measures: Free T</td>
<td>Free T levels significantly positively correlated with spatial ability (Spatial Composite: MRT and PFT composite score) in females (only in right-handed females).</td>
</tr>
<tr>
<td>Janowsky et al (1998)</td>
<td>30 healthy females</td>
<td>29.8</td>
<td><strong>Longitudinal study design with two test sessions during mid-luteal phase (with a gap of four weeks)</strong>&lt;br&gt;<strong>Clinical measures:</strong> Geriatric Depression Scale <strong>Cognitive measures:</strong>&lt;br&gt;General intelligence- Vocabulary subtest of Wechsler Adult Intelligence Scale (WAIS)&lt;br&gt;Spatial ability- Block Design test (WAIS-R), Card Rotation task.&lt;br&gt;Verbal and visuospatial memory- Toy Task Executive function- verbal fluency measured by Letter Fluency and Category Fluency Task. Psychomotor speed-Grooved Pegboard Test to assess fine motor dexterity and speed and Dart Throwing to assess visual-motor skills. Androgen measures: Free T.</td>
<td>No significant correlations between Free T levels and visuospatial or verbal ability across both sessions. Psychomotor speed (Dart-throwing performance) significantly positively correlated with Free T levels in females using their dominant hand (significantly negatively correlated with use of non-dominant hand).</td>
</tr>
<tr>
<td>Neave et al (1999)</td>
<td>14 heterosexual and 14 homosexual females</td>
<td>Heterosexual group-31, Homosexual group-26.5</td>
<td><strong>Cross-sectional study design</strong>&lt;br&gt;<strong>Cognitive measures:</strong>&lt;br&gt;Visuospatial ability- MRT, Water-Level Test.&lt;br&gt;Executive function- Verbal Fluency Test, Verbal Associations Test. Androgen measures: Free T</td>
<td>No significant association between Free T levels and cognitive function in both groups.</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean age (yrs)</td>
<td>Measures of Interest</td>
<td>Main Findings</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------</td>
<td>----------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hausmann et al (2000)</td>
<td>12 healthy females</td>
<td>29.1</td>
<td><em>Longitudinal study design with counterbalanced blood samples collected over 3-day intervals over 6 weeks</em>&lt;br&gt;<strong>Cognitive measures:</strong> Visuospatial ability- Revised Vandenberg and Kuse’s MRT (I and II), Mirrors Pictures Test (2D MRT), Hidden Figures Test. Androgen measures- T (unstated)</td>
<td>Stepwise multiple regression and partial correlation showed significant positive correlations between T levels and spatial ability (MRT) for both sessions.</td>
</tr>
<tr>
<td>Halari et al (2005)</td>
<td>42 healthy females</td>
<td>27.7</td>
<td><em>Cross-sectional study design&lt;br&gt;Cognitive measures:</em> General intelligence-Verbal Ability -Vocabulary subtest of WAIS-R&lt;br&gt;Verbal learning and memory and ability- Working Memory Task.&lt;br&gt;Visuospatial ability- Mental Rotations Test, Computerized Benton Judgement of Line Orientation Task.&lt;br&gt;Executive function- Phonological Fluency, Category Fluency.&lt;br&gt;Inhibition Task (with five forward and backward conditions)&lt;br&gt;<strong>Androgen measures:</strong> Total T, FAI, Free T (estimated from FAI).</td>
<td>No significant correlations (partial and curvilinear) between Total T or Free T levels and cognitive function.</td>
</tr>
<tr>
<td>Falter et al (2006)</td>
<td>22 healthy females and disembedding data of 12 females from another testing set</td>
<td>24.12</td>
<td><em>Cross-sectional study design&lt;br&gt;Cognitive measures:</em> Visuospatial ability: Shepard and Metzler’s MRT, Figure-disembedding Task (to measure the ability to find a smaller simpler form of a larger complex picture), Targeting Task (to measure speed of object-localisation) and Perceptual Discrimination Tasks (control) to measure object recognition ability.&lt;br&gt;<strong>Androgen measures:</strong> Free T</td>
<td>No significant correlation between Free T levels and cognitive function.</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean age (yrs)</td>
<td>Measures of Interest</td>
<td>Main Findings</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------------</td>
<td>----------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Burkitt et al (2007)         | 40 healthy females divided into high T ($n = 19$) and low T ($n = 18$) groups | 19.85          | Cross-sectional study design (with T levels measured before and after counterbalanced cognitive testing)  
  Cognitive measures:  
  Visuospatial ability: Vandenberg and Kuse’s MRT, Virtual Water Maze Task (vWM)  
  Androgen measures: Free T | Low Free T group performed significantly worse on Block 2 of Virtual Water Maze Task (average of second set of vWM) compared with high T group. |
| Schattmann & Sherwin (2007)  | 28 PCOS and 20 HC                      | PCOS = 27.7     | Cross-sectional study design  
  Clinical measures: POMS-Bipolar Form to measure mood.  
  Cognitive measures:  
  General intelligence - Vocabulary subtest of WAIS-III  
  Visuospatial ability- Vandenberg and Kuse’s MRT, Paper Folding Test, Water Level Test (Spatial perception)  
  Visuospatial Learning and Memory: Block-Tapping Task(forward and backward span)  
  Verbal learning and memory- RAVLT, Logical Memory, Paired Associates Test  
  Executive function- COWAT, Category Fluency, Digit Span (forwards and backwards),  
  Psychomotor speed- Finding A’s, Purdue Pegboard to measure perceptual speed and manual dexterity  
  Androgen measures:  
  Total T, Free T (estimated by FAI value), FAI | FAI levels significantly negatively correlated with psychomotor speed (total scores on Purdue Pegboard)  
  No other significant correlations between androgen levels and mood or cognitive function in PCOS and HC groups. |
| Gomez-Gil et al (2009)       | 33 FM transsexuals                    | Longitudinal-27.4  
  Cross-sectional-23.7  
  Off T treatment-23.7  
  On T treatment- 27.7 | Longitudinal (with a 6 month gap involving T treatment between testing sessions, $n = 14$) and cross-sectional study design (with patients on($n = 9$) and off ($n = 10$) T treatment for at least 6 months)  
  Cognitive measures:  
  Verbal memory- Logical memory Test (Wechsler Memory Scale-Revised)  
  Visuospatial memory- Visual Paired Associates Test (Wechsler Memory-Scale) and Rey-Osterrieth Complex Figure Test (ROCF).  
  Androgen measures: Total T, Free T | Significant improvement in one aspect of visuospatial memory (Visual Paired Associates 1-immediate recall) following androgen treatment over 6 months but no significant differences on another measure of spatial memory(ROCF performance) or verbal memory (Logical Memory Test).  
  In cross-sectional aspect of study, significantly better scores on visuospatial memory (Visual Paired Associates 1-immediate recall and ROCF: copy and delayed recall) in T group but no significant differences between groups on verbal memory. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Measures of Interest</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puts et al (2010)</td>
<td>160 healthy females</td>
<td>20.43</td>
<td><em>Cross-sectional study with a within-subjects design (involving 2 testing sessions)</em></td>
<td>Free T levels not significantly correlated with within-sex variation in spatial performance. Regression analyses showed Free T levels did not significantly predict spatial ability performance (MRT scores). Between-session improvement in MRT scores unrelated to Free T levels.</td>
</tr>
<tr>
<td>Durdiakova et al (2012)</td>
<td>9 healthy females</td>
<td>25.6</td>
<td><em>Cross-sectional study design (examining effect of cognitive task on T levels with 2 samples of T before and after MRT)</em></td>
<td>No correlation between baseline T levels and spatial ability (MRT total score) T levels significantly decreased following spatial task (MRT) in 8 of 9 females.</td>
</tr>
<tr>
<td>Barry et al (2013)</td>
<td>69 females with PCOS (14 hyperandrogenic and 37 non-hyperandrogenic) and 41 HC with comparable subfertility</td>
<td>PCOS-29 HC-35</td>
<td><em>Cross-sectional study design</em></td>
<td>Significant positive correlation between Total T levels and visuospatial ability (3-D MRT) in PCOS group (n = 56), not found in HC group (n = 30).</td>
</tr>
<tr>
<td>Romero-Martinez et al (2015)</td>
<td>24 Mothers of children with ASD (caregivers) and 22 non-caregivers (mothers of normal children) (HC)</td>
<td>45</td>
<td><em>Cross-sectional study design</em></td>
<td>Curvilinear relationship between Free T levels and verbal memory (RAVLT) in caregivers with increases in T levels significantly correlated with better verbal memory, up to a certain point followed by decreased performance with increasing T levels. In caregivers, high baseline Free T levels correlated with larger number of words remembered (better verbal memory performance) in List A Trial III. No significant associations found between Free T levels and other RAVLT measures. No correlation between Free T levels and cognitive function in HC group.</td>
</tr>
</tbody>
</table>
Table 5.3 Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean age (yrs)</th>
<th>Measures of Interest</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hussain et al. (2016)</td>
<td>45 healthy females</td>
<td>30.31</td>
<td>Cross-sectional study design</td>
<td>Total T levels not significant correlated with spatial or verbal memory.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cognitive measures:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>General intelligence- Test for Non-Verbal Intelligence-3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Verbal memory- RAVLT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Visuospatial ability- Virtual Navigation Task</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Androgen measures: Total T</td>
<td></td>
</tr>
<tr>
<td>Van Honk et al (1999)</td>
<td>16 healthy females</td>
<td>22</td>
<td>Cross-sectional study design with multiple salivary measurements of T preceding mood and cognitive assessment.</td>
<td>T sample collected six hours before assessment significantly positively correlated with facilitation interference (attending away from anger faces on the Emotional Stroop task). No significant association between T samples at other time points and emotion processing. No significant correlation between T levels (sampled at all three time points) and scores on depression rating scale (POMS).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinical measures:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>POMS, VAS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Emotion Processing measure: Face Stimulus Test; Facial expressions including task 1 with passive viewing of emotional faces and task 2 involving an oddball task while viewing anger and neutral faces.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Androgen measure: Free T</td>
<td></td>
</tr>
<tr>
<td>Stanton et al (2009)</td>
<td>14 healthy females</td>
<td>20.96</td>
<td>Cross-sectional study design</td>
<td>Regression analyses showed no significant correlation between Free T levels and amygdala or ventromedial prefrontal cortex BOLD response involved in emotion processing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinical measures:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fMRI to measure amygdala and ventromedial prefrontal cortex BOLD responses to anger faces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Emotion Processing measure:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Face Stimulus Test; Facial expressions including task 1 with passive viewing of emotional faces and task 2 involving an oddball task while viewing anger and neutral faces.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Androgen measure: Free T</td>
<td></td>
</tr>
</tbody>
</table>

ABS- Affect Balance Scale, A-dione-Androstenedione, A-diol- Androstenediol ,BDI- Beck Depression Inventory, Bioactive T = Biologically Active Testosterone, BMI = Body Mass Index, BSA- Brief Scale for Anxiety, CAB-S- Comprehensive Ability Battery Space Test, CES-D- Centre for Epidemiological Studies- Depression Rating Scale, COBAT- Controlled Oral Word Association Test, DEFT- Dynamic Facial Expression Task, DHEA= Dehydroepiandrosterone, DHEA-S=Dehydroepiandrosterone Sulfate, DSI- DeRogatis Symptom Inventory, FAI- Free Androgen Index, Free T- Free Testosterone, GHQ-12- General Health Questionnaire, HC = healthy controls, HRM = hormone group, HADS- Hospital Anxiety and Depression Scale, HAM-A- Hamilton Rating scale for Anxiety, HAM-D- Hamilton Depression Rating Scale, ICD-10- International Classification of Diseases, MAACL- Multiple Affect Adjective Check List, MADRS- Montgomery-Asberg Depression Rating Scale, MINI- Mini International Neuropsychiatric Interview, MMPI- Minnesota Multiphasic Personality Inventory, MRT- Mental Rotations Test, PRIME- Primary Care Evaluation of Mental Disorders Patient Health Questionnaire, PCOS-Q- Polycystic Ovarian Syndrome Questionnaire, POMS- Profile Of Mood States, PCOS = Polycystic Ovarian Syndrome, PL = placebo group, PMA- Primary Mental Abilities Test, QIDS- Quick Inventory of Depressive Symptomatology, ROCF-Rey-Osterrieth Complex Figure Test, RMET- Reading the Mind in the Eyes Task, SCL-90-R- Symptom Checklist Survey 90 R, SCID-I- Structured Clinical Interview for DSM-IV, STA-I- State-Trait Anxiety Inventory, Total T = Total Testosterone, VAS- Visual Analogue Scale, vWin- Virtual Water Maze Task, WAIS- Vocabulary subtest of Wechsler Adult Intelligence Scale, ZARS- Zung Anxiety Rating Scale, *= Insufficient information about the study sample provided by the authors – contact with corresponding author of the study was attempted at least twice, with no response.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean Age (yrs)</th>
<th>Intervventional studies: Measures of interest and study design</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Van Goozen et al (1994) | 22 female-to-male (FM) transsexuals | 25.7 | *Longitudinal study design with cross sex hormone treatment involving T administration for 3 months*  
*Clinical measures:*  
General Intelligence (verbal ability) measured by Verbal Reasoning Test  
Visuospatial ability - Rotated Figures Test,  
Executive function - Verbal Fluency measured by Word Production and Sentence Production Test. | Significant improvement in visuospatial ability (Rotated Figures Test), worse executive function (verbal fluency) and no significant change in verbal ability following T administration. |
MF-32.4  
HCF-29.6  
HCM-29 | *Longitudinal study design involving cross sex hormone treatment (T or oestrogen administration) with two test sessions separated by 12 weeks*  
*Clinical measures:* Diary for measuring mood  
*Cognitive measures:*  
General Intelligence (verbal ability) measured by Verbal Reasoning Test.  
Visuospatial Ability-Card Rotation Task  
Executive Function- verbal fluency- Word Production and Sentence Production Test. | Significantly better visuospatial ability (Card Rotation) and worse executive function (verbal fluency-Word Production) performance following T administration in FM group.  
Significantly decreased spatial ability performance and improved verbal fluency (Sentence production) in MF transsexuals following anti-androgen treatment.  
No changes in mood (assessed by Diary) post-treatment in both groups. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean Age (yrs)</th>
<th>Interventional studies: Measures of interest and study design</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Slabbekoorn et al (1999)  | 25 FM transsexuals and 20 MF         | MF- 29.1 FM- 26 | *Longitudinal study design involving cross-sex hormone treatment (androgen and antiandrogen (Cyproterone Acetate CPA)) with oestrogen treatment with testing sessions separated by baseline, 3, 12, and 18 months.*  
Cognitive measures:  
General Intelligence (verbal ability) measured by Verbal Reasoning Test  
Visuospatial ability – Rotated Figures (2-D and 3-D), Hidden Figures Task.  
Executive Function- verbal fluency- Words (VF-W) Task (adapted from Gordon et al., 1986) ,Sentences( FV-S) Task  
Psychomotor speed- Fine Motor Movement Task to measure manual dexterity and Perceptual Speed (Test D2) Task.                                                                                                                                                                                                                                                                                      | T administration (after a gap of 18 months) followed by improved spatial ability (3-D Rotated Figures and Hidden Figures task), no deteriorating effect of T administration on verbal fluency or psychomotor speed in FM transsexuals.  
Anti-androgen treatment followed by no significant changes in spatial ability and verbal fluency or psychomotor speed in MF transsexuals.                                                                                                                                                                                                |
| Postma (2000)             | 15 healthy females divided into two groups: placebo - T administration (n = 8) and T-placebo(n = 7) | 23.6            | *Double-blind placebo-controlled counterbalanced cross-over study design involving 0.5 mg T administration separated by four weeks.*  
Cognitive measures:  
Visuospatial learning and memory- Spatial Memory Stimulus with 3 conditions: Object-Location Memory (three conditions to measure selective aspects of memory: Positional Reconstruction, Object-to-position assignment, and Combined Condition Tasks).                                                                                                                                                                                                                           | In the first two conditions (Object-to-position and the Positional Reconstruction condition), the effect of T administration, delay of recall and interaction effect between T and delay of recall not significant.  
Significantly better performance in combined condition (after three-minute delay in recall) following T administration compared with placebo  
Separate t-tests for immediate and delayed recall scores showed no significant difference between T and placebo condition.                                                                                                                                                                                                                                    |
<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean Age (yrs)</th>
<th>Interventional studies: Measures of interest and study design</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Goozen et al (2002)</td>
<td>19 FM, 22 MF, 23 HC females, 20 HC males</td>
<td>FM- 26.2 MF- 31.4</td>
<td><em>Longitudinal study design involving cross-sex hormone treatment with androgen or antiandrogen (CPA with Estradiol) treatment (over a span of 14 weeks)</em>&lt;br&gt; <em>Cognitive measures:</em>&lt;br&gt; General intelligence (verbal ability) measured by Verbal Reasoning Test&lt;br&gt; Visuospatial ability- Line Orientation Test, Rotated Figures (RF) (2-D and 3-D), Targeted Throwing (spatial-motor) Task.</td>
<td>No change in visuospatial ability following androgen or anti-androgen treatment in either group.</td>
</tr>
<tr>
<td>Aleman et al (2004)</td>
<td>26 healthy females: T-placebo (n = 14) and placebo-T (N = 12) administration</td>
<td>Age range- 20- 32</td>
<td><em>Double-blind placebo-controlled cross-over study design with 0.5 mg T administration</em>&lt;br&gt; <em>Cognitive measures:</em> Visuospatial ability - Vandenberg and Kuse’s 3-D MRT (including four test drawings, where subjects are asked to identify two of the four test drawings depicted the target drawing in rotated positions).</td>
<td>Significantly improvement in visuospatial ability (MRT) following single T administration compared with placebo condition. Significant difference between T and placebo condition with better MRT scores for placebo-T but not T-placebo condition.</td>
</tr>
<tr>
<td>Miller et al (2005)</td>
<td>33 anorexic females with relative T deficiency divided into two groups- T (n = 24) and placebo (n = 9)</td>
<td>T - 25 Placebo-22</td>
<td><em>Randomized double-blind placebo-controlled trial with T administration (0.15 mg or 0.3 mg daily) for 3 weeks.</em>&lt;br&gt; <em>Clinical measures:</em> BDI&lt;br&gt; <em>Cognitive measures:</em> Visuospatial ability- Vandenberg and Kuse’s 3-D MRT&lt;br&gt; <em>Androgen measures:</em> Total T, Free T, DHEA-S.</td>
<td>At baseline, significant negative correlation between Free T levels and depression (BDI) and significant positive correlation between Free T levels and spatial ability (3-D MRT). Significant improvement in visuospatial ability following T administration compared with placebo condition. Significant improvement in mood in T group following 3 weeks of T administration compared with placebo which showed no change. Forward stepwise regression analysis showed T and placebo condition to be the only significant factors to predict change in depression scores (BDI).</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean Age (yrs)</td>
<td>Interventional studies: Measures of interest and study design</td>
<td>Key findings</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>---------------</td>
<td>----------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Schattmann &amp; Sherwin (2007)</td>
<td>19 hirsute females with PCOS randomly assigned to receive anti-androgen treatment (AA) ($n=8$) and placebo ($n=11$)</td>
<td>Placebo-26.8 Treatment-26.6</td>
<td>Randomised double-blind placebo-controlled study design involving anti-androgen administration (50 mg Cyproterone acetate (CPA) and oestrogen for 3 months) Clinical measures: POMS-Bipolar Form Cognitive measures: Visuospatial ability- Mental Rotations, Paper Folding (spatial visualisation), Water Level (spatial perception) Test Verbal ability- Vocabulary subtest of WAIS-III Verbal learning and memory- Rey Auditory Verbal Learning Test (RAVLT), Logical Memory Test, Paired Associates Test Executive function- verbal fluency measured by COWAT, Category Fluency Test, Digit Span (forwards and backwards), Block-Tapping (forward and backward span) Test Psychomotor speed- Finding A’s, Purdue Pegboard Androgen measures: Total T, Free T, FAI.</td>
<td>Significant improvement in verbal fluency (COWAT) performance following anti-androgen treatment in PCOS females, however, follow-up (lower) FAI levels unrelated to COWAT performance. No significant change in visuospatial ability, executive function (Block Tapping) verbal memory, psychomotor speed (manual dexterity and speed) following anti-androgen treatment. Post-treatment FAI levels significantly negatively correlated with spatial visualisation (Paper Folding test) and verbal learning and memory (Paired Associates test). No significant differences in mood between groups or across testing times.</td>
</tr>
<tr>
<td>Pintzka et al (2016)</td>
<td>42 healthy females</td>
<td>T-23.2 Placebo-21.8</td>
<td>Randomised double-blind placebo-controlled study design with 0.5 mg of T administration Cognitive measures: Visuospatial learning and memory -Virtual Environment (VE) Learning and Navigating Task, Sense of Direction Questionnaire Short-Form to measure navigational strategies Visuospatial ability-Vandenberg and Kuse’s MRT, fMRI stimuli with a self-paced Block Design Task. Androgen measures: Total T, A-dione, DHEA-S.</td>
<td>T group performed significantly better on spatial ability (MRT) and showed significantly better visuospatial learning (representation of direction (VE)) compared with placebo, however, no change following T administration on navigation ability (VE task).</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean Age (yrs)</td>
<td>Interventional studies: Measures of interest and study design</td>
<td>Key findings</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------</td>
<td>----------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>van Honk et al (2005)</td>
<td>16 healthy females</td>
<td>Age-range-19-26</td>
<td>Randomised double-blind placebo-controlled cross-over study design with 0.5 mg T administration (two days apart) Clinical Measures: STAI, POMS, Carver and White’s Behavioural Inhibition Scale (BIS) to measure short-term changes in anxiety Emotion processing measure: Masked Emotional Stroop Task (Pictures of Facial Affect) to measure unconscious emotional response to fearful faces.</td>
<td>Vigilant emotional response to masked fearful faces observed in placebo condition. Significant decrease in attentional bias for unconsciously seen (masked) fearful facial expressions following T administration compared with placebo condition. No change in response to happy faces. No significant difference in self-reported anxiety (STAI) and mood (POMS) between placebo and T conditions.</td>
</tr>
<tr>
<td>Van Honk et al. (2007)</td>
<td>16 healthy females</td>
<td>Age range-19-26</td>
<td>Double-blind within-subjects placebo-controlled cross-over study design with 0.5 mg T administration or placebo Clinical Measures: POMS Emotion Processing measure: Emotion-Recognition Task</td>
<td>Testosterone administration followed by reduced sensitivity to consciously recognising anger faces compared with placebo. No significant effect of testosterone on faces of fear or disgust. No significant difference between testosterone and placebo conditions on mood.</td>
</tr>
<tr>
<td>Hermans et al (2007)</td>
<td>20 healthy females</td>
<td>Age-range-18-23</td>
<td>Randomised double-blind placebo-controlled mixed-factorial cross-over study design with 0.5 mg T administration Clinical measures: STAI (Trait) and the computerised version of POMS (for measuring mood and anxiety) and VAS of the Self-Assessment Manikins (to measure subjective experience of the stimuli such as valence and arousal), skin conductance and cardiac responses. Emotion processing measure: International Affective Picture System photoset with emotional content (including three valence categories: negative, neutral and positive)</td>
<td>Lower skin conductance responses and reduced affective startle response to negative valence pictures in high-trait anxiety participants following T administration. No association between T administration and mood.</td>
</tr>
<tr>
<td>Study</td>
<td>Study sample</td>
<td>Mean Age (yrs)</td>
<td>Interventional studies: Measures of interest and study design</td>
<td>Key findings</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------------------------------------</td>
<td>----------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>van Wingen et al (2009)</td>
<td>42 healthy females divided into two groups-Experimental group: 25 middle-aged females with lower androgen levels and lower amygdala reactivity. HC:17 younger females</td>
<td>Experimental group = 42 HC = 23</td>
<td><strong>Double-blind placebo controlled cross-over study design involving 0.9 mg T administration (in middle-aged females) with two test sessions</strong>&lt;br&gt;<strong>Clinical measures:</strong> fMRI to measure amygdala response during facial emotional face matching task, Mood Rating Scale and STAI to assess mood and anxiety.&lt;br&gt;<strong>Emotion processing measure:</strong> Blocked Design Task including an emotional and visuo-motor control condition.&lt;br&gt;<strong>Androgen measures:</strong> Total T, DHEA-S, DHT.</td>
<td>Increased amygdala reactivity following T administration in experimental group during emotional condition compared with visuo-motor control condition of behavioural task. No significant effect on accuracy of responses or reaction time on emotion processing (behavioural task) following T administration.&lt;br&gt;T levels not correlated with mood, anxiety or cognitive function.</td>
</tr>
<tr>
<td>Van Honk et al (2011)</td>
<td>16 healthy females</td>
<td>21</td>
<td><strong>Double blind placebo-controlled within-subjects cross-over study design with 0.5 mg T administration (or placebo with a 48 hr gap)</strong>&lt;br&gt;<strong>Clinical measures:</strong> POMS&lt;br&gt;<strong>Emotion processing measure:</strong> Reading the Mind in the Eyes Test (RMET) (computerised adaptation) to assess facial emotion processing&lt;br&gt;<strong>Androgen measures:</strong> Free T</td>
<td>T administration followed by significant decrease in facial emotion processing (RMET) compared with placebo. Significant negative correlation between Free T levels and RMET in placebo condition.&lt;br&gt;T levels unrelated to self-reported mood.</td>
</tr>
<tr>
<td>Terburg et al (2012)</td>
<td>20 healthy females</td>
<td>Age-range 20-25</td>
<td><strong>Longitudinal placebo-controlled counterbalanced study design with 0.5mg T administration for one week.</strong>&lt;br&gt;<strong>Clinical measures:</strong> POMS&lt;br&gt;<strong>Emotion processing measure:</strong> Social dominance task including 30 stimuli including angry, happy and neutral facial expressions</td>
<td>Significant reduction in gaze aversion (slower gaze aversion/higher attendance) related to unconsciously shown anger faces following T administration compared with placebo condition. No effect of T administration on self-reported mood states.</td>
</tr>
<tr>
<td>Bos et al. (2013)</td>
<td>12 healthy females</td>
<td>20.4</td>
<td><strong>Double-blind placebo-controlled counterbalanced cross-over design with T administration (0.5mg)</strong>&lt;br&gt;<strong>Clinical measures:</strong> fMRI to measure response to emotional expressions, STAI.&lt;br&gt;<strong>Emotion processing measure:</strong> Dynamic Facial Expression Task (DEFT), with fear (experimental condition) and happy (control condition) faces.</td>
<td>Increased and equal amygdala response to both fear and happy (control) faces (no selective effect on fear response) following T administration compared with placebo.&lt;br&gt;No association between baseline testosterone levels and amygdala response to fear faces.</td>
</tr>
</tbody>
</table>
### Table 5.4 Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Study sample</th>
<th>Mean Age (yrs)</th>
<th>Interventional studies: Measures of interest and study design</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Enter et al (2015)     | 18 females with Social Anxiety Disorder (SAD) and 19 HC. SAD- 23.1 HC- 25.2 | **Randomised double-blind placebo-controlled cross-over study design with 0.5 mg T administration on two separate days** | **Clinical measures:** MINI (screening), BDI and the Liebowitz Social Anxiety Scale. Gaze avoidance indexed by relative reduction of initial gaze fixations on the eye-region measured by eye-tracker  
**Emotion processing measure:** Face stimuli (NimStim set of facial expressions) including angry, happy and neutral facial expressions. | Lower baseline T levels in SAD group compared with HC.  
Significant increase in first fixations towards the eye-region of facial stimuli (reduced gaze avoidance, initially observed in placebo condition) in SAD group, not in HC following T administration.  
Significant effect of T on angry faces; not on other emotional expressions in the SAD group.  
No significant changes in emotion processing following either T or placebo condition in HC group. |
| Bos et al. (2016)      | 16 healthy females                                | 20.8           | **Randomised double-blind placebo-controlled counterbalanced cross-over study design with 0.5 mg T administration**               | Significant reduction in connectivity between the left inferior frontal gyrus (IFG) (activated during RMET) and the anterior cingulate cortex and the supplementary motor area following T administration during the facial emotion processing task (RMET) compared with placebo.  
No change in emotion processing (RMET) or self-reported mood (POMS) following T administration. |
| Olsson et al 2016      | 33 healthy females                                | 26.9           | **Randomised double-blind placebo controlled study design with T administration**  
**Emotion processing measure:** RMET                                                                  | Decreased accuracy in inferring emotional states (RMET) following T administration compared with placebo condition. |

*Key: SAD = Social Anxiety Disorder, HC = Healthy Controls, T = Testosterone, Bioactive T = Biologically Active Testosterone, BDI = Beck Depression Inventory, CES-D = Centre for Epidemiological Studies - Depression Rating Scale, MDS = Medical Outcomes Study - Social Functioning Scale, POMS = Profile of Mood States, ROCF = Rey-Osterreith Complex Figure Test, RMET = Reading the Mind in the Eyes Task, SCL-90-R = Symptom Checklist Survey 90 R, SCID-I = Structured Clinical Interview for DSM-IV, STAI = State-Trait Anxiety Inventory, T = Total Testosterone, VAS = Visual Analogue Scale, vWM = Virtual Water Maze Task, WAIS = Vocabulary subtest of Wechsler Adult Intelligence Scale, ZAI = Zung Anxiety Rating Scale.*

**Androgen measures:** Free T
5.3.1 Cross-sectional Studies Relating Mood/Anxiety to Androgen Levels in PCOS Samples

5.3.1.1 Study and patient characteristics

Twenty-one studies were identified. All studies examined mood in PCOS samples, and 14 of 21 studies included a healthy control group. Ten of 21 studies measured anxiety as well as symptoms of depression. Most studies with a control group reported higher levels of depression and/or anxiety in their PCOS group compared with healthy control participants, with the exception of two studies (Milsom, Nair, Ogilvie, Stewart, & Merry, 2013; Pastore, Patrie, Morris, Dalal, & Bray, 2011). All 21 studies used a cross-sectional design.

5.3.1.2 Symptoms of depression and androgen levels

**Free Testosterone and Free Androgen Index (FAI):** Free Testosterone and FAI levels were measured in 12 out of 21 studies.

Five studies found a significant association between FAI or Free Testosterone levels and mood (Bazarganipour et al., 2013; Cinar et al., 2011; Jedel et al., 2011; Moran, Deeks, Gibson-Helm, & Teede, 2012; Weiner et al., 2004). Three studies found an association between higher androgen (FAI) levels and higher symptoms of depression \( (n = 580) \), two others found a negative \( (n = 35) \) and curvilinear \( (n = 27) \) association respectively, while the remaining seven studies found no significant relationship between mood and androgen levels \( (n = 541) \).

Three studies (two using correlational analyses and one using comparative analysis) found a significant positive association between FAI levels and symptoms of depression. A large study including 226 females found that higher FAI levels were associated with greater depression severity measured by the Hospital Anxiety and Depression Scale (HADS) \( (r = 0.321, p < 0.05) \) but not with the Beck Depression Inventory (BDI) \( (n = 226) \) (Cinar et al., 2011). Similarly, Moran et al. (2012) found higher FAI levels (and age) to be significantly associated with greater
depression severity (HADS) (multivariate regression showed log-transformed FAI levels ($r = 0.38, p = 0.003$) to significantly predict depression levels) in a sample of 54 females with PCOS. In another large study (Bazarganipour et al., 2013) significantly higher FAI levels were observed in females with PCOS with symptoms of depression (HADS) ($13.15 \pm 46.99$) compared with females with PCOS but without symptoms of depression ($9.47 \pm 30.60$) ($p < 0.05$) (total $n = 300$).

In contrast, Jedel et al. (2011) found significantly lower Free Testosterone levels in PCOS females with higher depression rating-scale scores (Montgomery-Asberg Depression Rating Scale; MADRS $\geq 11$) ($n = 35$) compared with non-depressed PCOS females ($p = 0.025$) ($n = 37$). Regression analysis showed a significant negative correlation between Free Testosterone levels and mood (lower Free Testosterone levels associated with higher scores on MADRS).

Although Weiner et al. (2004) did not find a significant correlation between mood (State-Trait Depression Adjective Check List) and Free Testosterone levels in their PCOS group, a curvilinear relationship was found between mood and Free Testosterone levels across the entire sample including PCOS ($n = 27$) and healthy control females ($n = 27$). Highest mood symptom scores were found at mid-range of testosterone levels in this study.

Seven remaining studies ($n = 541$) did not find a significant relationship between Free Testosterone or FAI levels and mood (scales used comprised of the Structured Clinical Interview for DSM-IV (SCID-I), BDI, QIDS, PHQ9, MINI, CES-D) (Annagür et al., 2013; Hollinrake, Abreu, Maifeld, Van Voorhis, & Dokras, 2007; Klimczak, Szlendak-Sauer, & Radowicki, 2015; Livadas et al., 2011; Mansson et al., 2008; Pastore et al., 2011; Rasgon et al., 2003). This is in accord with a meta-analysis, which in a meta-regression, showed no relationship between depression in PCOS and Free Testosterone or FAI levels characteristic of PCOS (Cooney et al., 2017). In this analysis, no evidence was found that Free Testosterone/FAI levels were higher in women with PCOS and concurrent depression. However, this analysis did
not include all of the studies reviewed above since it excluded studies without a control group among other exclusion criteria which included: 1) studies that used self-report measures for PCOS symptoms, 2) studies which recruited adolescents with PCOS (included in the current review (Milsom et al., 2013)), 3) studies that did not report mean BMI in both groups, or, 4) studies that did not report prevalence of depression or anxiety but reported mean scores.

**Total Testosterone:** Total Testosterone was assessed by 19 out of 21 studies in this section, with only one study finding a significant association between Total Testosterone and symptoms of depression. Jedel et al. (2011) found lower Total Testosterone levels to be associated with greater depression severity (MADRS) in PCOS females with depression compared with PCOS females with lower MADRS scores ($n = 37$).

**DHEA/DHEA-S:** DHEA/DHEA-S levels were measured in 10 studies (Adali et al., 2008; Annagür et al., 2013; Asik et al., 2015; Hollinrake et al., 2007; Jedel et al., 2011; Klimczak et al., 2015; Livadas et al., 2011; Pastore et al., 2011; Rocco et al., 1991; Soyupek et al., 2008). Only one study found a significant association between mood and DHEA-S, with DHEA-S levels being significantly higher in PCOS females with MDD ($n = 23$) compared with non-depressed PCOS females ($n = 30$) (Annagür et al., 2013).

**A-dione:** Four studies measured A-dione levels (Jedel et al., 2011; Klimczak et al., 2015; Livadas et al., 2011; Rocco et al., 1991), and only Rocco et al. (1991) found a significant association between A-dione levels and mood. In this small study, higher A-dione levels were found in females with PCOS and depression (assessed by the Minnesota Multiphasic Personality Inventory; MMPI) ($n = 11$) compared with non-depressed PCOS females ($n = 10$) (Rocco et al., 1991).

In summary there is no consistent evidence of a direct relationship between androgen levels, particularly the key measures of Free Testosterone and/or FAI levels and mood in PCOS. Of
the twelve studies that measured Free Testosterone and FAI levels in association with mood, three large studies (of which two used correlational analyses, and one used comparative group (depressed vs non-depressed analysis) found a positive association (higher testosterone levels associated with higher symptoms of depression), two studies (one using comparative group and correlational analysis, the other using only correlational analysis) found a negative and a curvilinear relationship, however, seven others (with a mixture of comparative and correlational analyses) failed to find any significant results. It is to be noted that the sample size was approximately equal in the positive and negative (no significant findings) studies. Interestingly, studies that found a positive relationship used the HADS to assess symptoms of depression while the negative studies did not. The HADS was primarily developed to assess symptoms of depression in a medical sample such as PCOS, and it excludes items relating physical symptoms involved in medical conditions including appetite, tiredness/fatigue, and sleep disturbances which other depression rating scales such as the BDI include. Therefore, the HADS may be more likely to demonstrate a clear relationship between the psychological symptoms of depression and androgen levels in PCOS.

There is no strong evidence for significant associations between mood and Total Testosterone, which is expected since Total Testosterone is not a reliable measure of overall androgen levels, as compared with Free Testosterone or FAI levels. No convincing evidence exists regarding the relationship between mood and levels of precursor androgens including DHEA/ DHEA-S or A-dione. Only one study each including a PCOS sample found an association between DHEA-S and A-dione and mood respectively (Annagür et al., 2013; Rocco et al., 1991).

5.3.1.3 Androgen levels and anxiety symptoms in PCOS samples in cross-sectional studies

Twelve studies assessed anxiety in PCOS samples (Annagür et al., 2013; Barry, Kuczmiczyk, et al., 2011; Bazarganipour et al., 2013; Cinar et al., 2011; Hahn et al., 2005; Hollinrake et al., 2007; Jedel et al., 2011; Livadas et al., 2011; Mansson et al., 2008; Moran et al., 2012; Rocco
et al., 1991; Weiner et al., 2004). Six \((n = 805)\) of 12 studies found significant associations between androgen levels and anxiety levels (scales included the SCID-I, HADS, STAI, and MINI, with the most common scale being the STAI) (Annagür et al., 2013; Bazarganipour et al., 2013; Cinar et al., 2011; Livadas et al., 2011; Mansson et al., 2008; Weiner et al., 2004). Four large studies found a significant positive association between state anxiety and FAI levels \((n = 705)\) (Bazarganipour et al., 2013; Cinar et al., 2011; Livadas et al., 2011; Mansson et al., 2008), one small study found a negative correlation between state anxiety and FAI levels \((n = 27)\) (Weiner et al., 2004), one small study found a positive association between GAD and DHEAS levels \((n = 20)\) (Annagür et al., 2013) and six remaining studies \((n = 409)\) found no significant association (Barry, Kuczmierczyk, et al., 2011; Hahn et al., 2005; Hollinrake et al., 2007; Jedel et al., 2011; Moran et al., 2012; Rocco et al., 1991).

An increased prevalence of anxiety disorders, particularly GAD and SAD, has been reported in females with PCOS ([OR] 6.88 95% CI 2.5-18.9, 4 studies, (Dokras et al., 2012), \(p = 0.04\), (Laggari et al., 2009)). A high incidence of social anxiety in women with PCOS may be expected considering clinical features of acne, hirsutism, and weight gain which are involved in the syndrome and which may contribute to poor self-esteem, and sensitivity to social scrutiny (Barnard, Ferriday, et al., 2007; Hahn et al., 2005; Kitzinger & Willmott, 2002). Furthermore, anxiety and mood disorders are frequently comorbid conditions (Brawman-Mintzer, Emmanuel, Jarrell, & Ballenger, 1993), which may help explain increased anxiety prevalence in depressed females with PCOS. Further studies are needed to understand whether testosterone plays a role in directly influencing anxiety levels in females with PCOS.

Overall, Free Testosterone and/or FAI levels have been examined in relation to anxiety by six studies, of which four found significant positive associations, one found a negative association while the one remaining study found no significant association. In this study, although PCOS females with GAD \((n = 20)\) were found to have significantly higher DHEA-S levels compared
with PCOS females without GAD ($p = 0.001$) ($n = 30$) (Annagür et al., 2013), no significant association was found between Free Testosterone levels and anxiety levels. In a large study ($n = 300$ females with PCOS), significantly higher FAI levels were found in females with PCOS and symptoms of anxiety (HADS) ($p < 0.001$) ($n = 96$) compared with females with PCOS without symptoms of anxiety ($n = 204$) (Bazarganipour et al., 2013). Livadas et al. (2011) found higher FAI levels to correlate significantly with more severe state anxiety (STAI-S) in 130 females with PCOS with varying levels of anxiety. This positive correlation between FAI levels and state anxiety (STAI-S) was replicated in another large sample ($n = 226$) of females with PCOS (Cinar et al., 2011). In contrast, Weiner et al. (2004) reported a significant negative correlation between Free Testosterone levels and state anxiety (STAI-S) in females with PCOS ($n = 27$). In their healthy group, however, a positive correlation between Free Testosterone levels and state anxiety was reported ($n = 27$). One study examining FAI levels in relation to SAD found higher FAI levels in PCOS females with SAD ($n = 49$) compared with PCOS females without SAD ($n = 49$) (Mansson et al., 2008).

The remaining six studies that measured anxiety in relation to Total and Free Testosterone levels found no significant association between anxiety and testosterone levels (total $n = 446$) (Barry, Kuczmierczyk, et al., 2011; Hahn et al., 2005; Hollinrake et al., 2007; Jedel et al., 2011; Moran et al., 2012; Rocco et al., 1991). It is to be noted that three of six studies finding no association between anxiety and testosterone levels assessed only Total Testosterone levels (Barry, Kuczmierczyk, et al., 2011; Hahn et al., 2005; Rocco et al., 1991). Anxiety rating scales used by the negative studies included the HADS (Zigmond & Snaith, 1983), SCL-90-R (Derogatis & Fitzpatrick, 2004), PRIME-MD PHQ (Spitzer, Kroenke, & Williams, 1999), the Comprehensive Psychopathological Rating Scale for Affective Syndrome (Svanborg & Åsberg, 1994), and the STAI (Spielberger, 2010). The STAI was used by only one negative study, however, the positive studies that found an association between anxiety and androgen levels...
tended to commonly use the STAI together with the HADS to assess symptoms of anxiety and tended to assess FAI levels.

Overall, studies that did find a significant positive association were larger (total $n = 705$) compared with studies that did not find an association (total $n = 446$). SAD is of interest given emotional processing data, which will be discussed in Section 5.4.

### 5.3.1.4 Overall summary

There is no consistent evidence of a direct relationship between androgen levels and mood and/or anxiety in PCOS. Overall, studies that found a positive association between higher FAI levels and greater symptoms of depression used the HADS as the primary depression rating scale, whereas the studies that found a negative association or found no significant association used other depression rating scales. Since the HADS is more appropriate to assess symptoms of mood and anxiety in medical samples, such as PCOS, and is a recognised and well-validated measure, findings of positive associations between testosterone levels and higher symptoms of mood in studies using the HADS could be considered as more reliable. A meta-analytic review of a smaller number of studies (Cooney et al., 2017) which measured mood and anxiety in PCOS and measured Free Testosterone or FAI levels and had a control group demonstrated higher Free Testosterone/FAI levels in females with PCOS and concurrent anxiety ($p < 0.05$), but no relationship between testosterone levels and mood was reported. However, in Cooney et al’s (2017) study, only 13 studies assessed androgen levels in relation to symptoms of mood and/or anxiety, which were included in the current study. Apart from these 13 studies (Annagür et al., 2013; Asik et al., 2015; Bazarganipour et al., 2013; Bhattacharya & Jha, 2010; Cinar et al., 2011; Hollinrake et al., 2007; Jedel et al., 2010; Klimczak et al., 2015; Livadas et al., 2011; Mansson et al., 2008; Moran et al., 2012; Rahiminejad et al., 2014; Soyupek et al., 2008), the current review included eight additional cross-sectional studies including PCOS samples (Adali et al., 2008; Barry, Kuczmiarczyk, et al., 2011; Hahn et al., 2005; Milsom et al., 2013;
Pastore et al., 2011; Rasgon et al., 2003; Rocco et al., 1991; Weiner et al., 2004) which led to somewhat different results from Cooney et al’s (2017) review regarding the specific relationship between mood, anxiety and androgen levels. As noted previously (see page 118), Cooney et al. (2017) had a different exclusion criteria from the current review. Therefore, in the current review, different findings related to the association between Free Testosterone levels and mood/anxiety may have emerged due to broader inclusion criteria. In the current study, a meta-analysis was not carried out since most studies included in the current review did not have equivalent measures and used different methodological procedures. Therefore, a systematic review was considered more appropriate.

5.3.2 Studies relating mood/anxiety to androgen levels in non-PCOS samples (Cross-sectional and interventional findings)

Twelve studies assessed the association between androgen levels and symptoms of depression and/or anxiety in females without PCOS (see Table 5.2). The five experimental/interventional studies included in this section involved: antidepressant treatment (sertraline, clomipramine) (Baischer et al., 1995; Kumsar et al., 2014), DHEA treatment (Wolkowitz et al., 1999), testosterone administration in HIV-infected patients (Dolan Looby, Collins, Lee, & Grinspoon, 2009), and testosterone administration in healthy females (Schutter, Peper, Koppeschaar, Kahn, & van Honk, 2005), to examine the effects on mood and anxiety.

5.3.2.1 Depression and androgen levels in non-PCOS females (Cross-sectional findings)

Free Testosterone and FAI (including Bioactive Testosterone and Bioavailable Testosterone): Free Testosterone and/or FAI levels were assessed in seven out of 12 studies, with three cross-sectional studies (total n = 71) (including females with hirsutism and females with Borderline Personality Disorder) finding a significant positive correlation between Free Testosterone and FAI levels and greater symptoms of depression) (Derogatis, Rose, Shulman,
& Lazarus, 1993; Roepke et al., 2010; Shulman et al., 1992), two studies (total $n = 95$) (one including Sertraline treatment, the other cross-sectional study including females with anorexia nervosa) finding a negative association (higher Free Testosterone and/or FAI levels associated with less severe symptoms of depression) (Kumsar et al., 2014; K. Miller, Wexler, et al., 2007) while the remaining two (including one experimental study with HIV females and the other cross-sectional study including healthy females) (total $n = 101$) (Dolan Looby et al., 2009; Schutter, Meuwese, Bos, Crone, & Peper, 2017) found no significant association between Free Testosterone/FAI levels and mood.

Roepke et al. (2010) found a significant positive correlation between symptoms of depression (HAM-D) and FAI levels in females with BPD ($n = 31$). However, this correlation was not significant after controlling for BMI. The two other studies recruiting females with hirsutism showed more severe symptoms of depression (DeRogatis Symptoms Inventory and Affect Balance Scale) to be significantly correlated with higher Free Testosterone levels (total $n = 40$). Significant positive correlations between worse mood (lower positive affect) and higher Free Testosterone and Bioactive Testosterone were also found in both studies ($r = 0.60, p <0.01$ for both studies) (Derogatis et al., 1993; Shulman et al., 1992).

In contrast, two out of seven studies found lower Free Testosterone levels to be associated with more severe symptoms of depression (Kumsar et al., 2014; K. Miller, Wexler, et al., 2007). In a sample of 43 females with anorexia and depression, a significant negative correlation was found between Free Testosterone levels and depression severity (lower Free Testosterone levels associated with higher HAM-D scores) (K. Miller, Wexler, et al., 2007). Multivariate regression analysis found Free Testosterone levels to be positively correlated with depression severity. However, a group with anorexia nervosa would be likely to show significant variation in overall hormone levels. In Kumsar et al’s (2014) interventional study involving antidepressant treatment (Sertraline) over a six-week period, baseline FAI levels were found to be significantly
lower in females with depression ($n = 52$) compared with healthy control participants ($p < 0.001$) ($n = 30$). A significant increase in testosterone levels was noted following the six-week antidepressant treatment in the patient group, and post-treatment levels were not significantly different between the two groups.

The remaining two studies including female patients with HIV ($n = 25$) (Dolan Looby et al., 2009) and healthy females ($n = 76$) (Schutter et al., 2017) found no significant correlation between Free Testosterone or FAI levels and mood. Dolan Looby et al’s (2009) study included females with HIV who showed lower testosterone levels at baseline compared with healthy females, however, this study found no significant association between testosterone levels and mood.

To summarise, of the seven studies that measured Free Testosterone levels and FAI levels in non-PCOS samples, three found higher Free Testosterone or FAI levels to be associated with greater depression severity, two found lower Free Testosterone and FAI levels to be associated with greater depression severity, and the remaining two found no significant relationship between Free Testosterone and depression severity. It is to be noted, however, that two of the three studies that found a positive association between testosterone levels and mood in non-PCOS samples used tests not specifically designed to measure current severity of symptoms of depression (DeRogatis Symptoms Inventory and Affect Balance Scale), which may be relatively insensitive to clinically significant symptoms of depression. Overall, the results are inconsistent.

**Total Testosterone:** Total Testosterone levels were measured in seven out of 12 studies. Two studies reported a negative association between Total Testosterone and depression severity. Miller et al. (2007) found Total Testosterone levels to be significantly negatively correlated with depression (lower Total Testosterone associated with higher depression scores) in females with anorexia ($n = 43$). Kumsar et al. (2014) found lower Total Testosterone levels in an
untreated depressed group \((n = 52)\) compared with a non-depressed group \((n = 30)\). One study out of seven reported a significant positive association between Total Testosterone and mood, with higher Total Testosterone levels found in a sample of 20 untreated females with depression, compared with a healthy control group \((n = 10)\) (Baischer et al., 1995). The remaining four studies found no significant relationship between Total Testosterone levels and mood in varying groups (see Table 5.2) (Derogatis et al., 1993; Dolan Looby et al., 2009; Roepke et al., 2010; Shulman et al., 1992).

**DHEA, DHEA-S and A-dione:** DHEA and DHEA-S levels were measured in four cross-sectional studies. No significant relationship between DHEA or DHEA-S and mood was found in any of these studies (Derogatis et al., 1993; Landén et al., 2004; Roepke et al., 2010; Shulman et al., 1992). A-dione was measured in two cross-sectional studies, however, no significant relationship with mood was reported (Roepke et al., 2010; Shulman et al., 1992).

5.3.2.2  *Depression and androgen levels in non-PCOS females:* (Interventional findings)

Three small interventional studies examined the effect of androgen administration on mood; one in a sample of healthy females (Schutter et al., 2005), one in a sample of depressed females (Wolkowitz et al., 1999) and one in females with HIV (Dolan Looby et al., 2009). It is important to understand that results from experimental studies involving testosterone administration are quite different from results from studies involving anti-androgen medication, since the latter comprise of a completely different methodology in a medical sample with hormonal abnormalities. Additionally, it is not reasonable to suggest that testosterone administration studies are likely to have the opposite results of anti-androgen administration. The former is often short-term and testosterone levels increase for only a short period of time. It is clearly not just the opposite of treatment for PCOS. However, these studies can provide useful data regarding the effects of androgens in the brain.
A randomised placebo-controlled trial showed significant improvement in mood symptoms (BDI) in a sample of females with HIV and with lower baseline testosterone levels \((n = 13)\) following testosterone administration over 18 months, compared with the placebo condition \((-6.8\pm2.2 \text{ vs. } -1.9\pm3.1; p =0.02)\), \((n = 12)\) (Dolan Looby et al., 2009). Similarly, in another small randomised, placebo-controlled study involving DHEA-S treatment for females with depression (Wolkowitz et al., 1999), a significant decrease in HAM-D scores (improvement in mood) was found in depressed females \((n = 5)\) compared with placebo \((n = 5)\) following DHEAS treatment over six weeks (group-by-time interaction: \(F = 5.21, \text{df} = 1, 20, p < 0.04\)).

This result may be more likely related to the anti-glucocorticoid effects of DHEA (Gallagher et al., 2008; Kalimi, Shafagoj, Loria, Padgett, & Regelson, 1994) which is in line with research showing that cortisol suppression helps to improve low mood (Murphy, 1991) (see Chapter 2). However, in another small randomised double-blind placebo-controlled crossover study \((n = 14)\), testosterone administration was not associated with significant change in self-reported mood or anxiety (POMS) compared with the placebo condition (Schutter et al., 2005). It is important to note that the POMS is not a depression rating scale and is suitable for assessing six different dimensions of mood swings over a short period of time, therefore, the results may not accurately reflect symptoms of depression.

Two other interventional studies involved antidepressant treatment and found contrasting results despite using similar antidepressant agents (Clomipramine and Sertraline). At baseline, a positive association was found in one study, with significantly elevated Total Testosterone levels found in untreated depressed patients \((n = 20)\) compared with controls \((n = 10)\) (Baischer et al., 1995). Following Clomipramine treatment (four weeks), Total Testosterone levels were found to reduce significantly in the patient group, to the point that they were not significantly different from healthy controls. In contrast, in a larger sample, Kumsar et al. (2014) found lower pre-treatment Total Testosterone and FAI levels in depressed patients \((n = 52)\) compared with healthy females \((n = 30)\). A significant increase in androgen levels was found following
antidepressant treatment (Sertraline over a course of six weeks) in females with depression. No significant differences were observed between the two groups post-treatment.

5.3.2.3 Overall summary

To summarise, the association between Free Testosterone or FAI levels and mood in non-PCOS samples remains unclear, with studies showing mixed findings in disparate groups. Total Testosterone does not seem to be correlated with symptoms of depression in non-PCOS samples with anorexia nervosa and depression, with only one study finding a positive association in untreated patients with depression while the remaining studies failed to find an association. There is no evidence of a significant relationship between DHEA/DHEA-S and A-dione levels and mood in non-PCOS samples.

5.3.2.4 Anxiety and androgen levels in non-PCOS samples

Of the seven studies that measured anxiety in non-PCOS samples, three found no significant relationship between androgen levels and anxiety (n = 110) (Schutter et al., 2017; Schutter et al., 2005; Shulman et al., 1992). The remaining four studies found significant relationships between anxiety levels and androgens, with three finding a negative association (n = 175) and one a positive association (n = 20).

Two studies (total n = 144) including females with anorexia nervosa and females with epilepsy and anxiety, respectively, reported a negative association with lower androgen levels significantly related to greater symptoms of anxiety (HAM-A, HADS, Zung Anxiety Rating Scale (ZARS) (K. Miller, Wexler, et al., 2007; Zheleznova et al., 2013). In the study of females with epilepsy and anxiety, however, the type of testosterone measured was not specified (e.g., Total or Free) (Zheleznova et al., 2013). Additionally, Derogatis et al. (1993) found Free Testosterone and Bioactive Testosterone levels to be significantly associated with greater phobic anxiety (De Rogatis Symptom Inventory [DSI]) in 20 hirsute patients. One study found
lower Total Testosterone levels in individuals with SAD (assessed by DSM-IV based self-report questionnaire) \( (n = 31) \) compared with healthy controls \( (n = 185) \) (Landén et al., 2004). As with depression studies, measures of anxiety including HAM-A, Zung Anxiety Rating Scale (ZARS), a DSM-IV based self-report questionnaire and the DSI were variable in terms of their sensitivity in detecting anxiety symptoms at mild and severe ends of the spectrum. The DSM-IV based self-report questionnaire used in Landén et al’s (2004) was not specifically an anxiety rating scale and covered an assessment of social anxiety disorder and five additional psychiatric conditions including mood, panic attacks, obsessive compulsive disorder, and premenstrual dysphoric disorder. The DSI is an 89-item self-report inventory and was designed to measure overall psychiatric distress over several dimensions, including panic anxiety, interpersonal sensitivity, depression, and phobic anxiety. The ZARS is designed to measure anxiety levels within the normal range extending up to levels of extreme anxiety. However, one study found that females showed higher scores on the ZARS (and the STAI) compared with males and that scores were inversely correlated with age (Knight, Waal-Manning, & Spears, 1983). The HAM-A is a suitable measure for detecting the severity of symptoms of anxiety and their change over time. However, the study that assessed anxiety in samples with epilepsy using the HAM-A did not assess the type of testosterone measured, thus likely to affect results (Zheleznova et al., 2013). This result including non-significant associations between anxiety and testosterone levels in non-PCOS samples are in contrast to findings in PCOS samples involving positive associations between FAI levels and symptoms of anxiety. It is worth considering the range of testosterone levels that individuals show at baseline (for example significantly elevated in PCOS vs low in females with anorexia or HIV) (Dolan Looby et al., 2009; K. Miller, Lawson, et al., 2007) in relation to symptoms of anxiety. Overall, these results do not suggest a strong association between testosterone levels and symptoms of anxiety.
5.3.2.5 Methodological issues in studies examining the association between symptoms of mood and androgen levels in PCOS and non-PCOS samples

5.3.2.5.1 Sampling bias

An explanation for an increased rate of symptoms of depression in the PCOS samples may simply relate to a sampling bias. Patients with PCOS displaying physical/visible symptoms (including hair and skin abnormalities and/or infertility) are more likely to experience psychiatric symptoms as a result of the physical symptoms of PCOS, compared with females without physical symptoms of PCOS (Asik et al., 2015; Deeks et al., 2010; Dunaif, 1997; Elsenbruch et al., 2006; Ferriman & Gallwey, 1961; Kitzinger & Willmott, 2002; Mechanick & Dunaif, 1990; Moran, Gibson-Helm, Teede, & Deeks, 2010; Polson et al., 1988; Sonino et al., 1993). Since females with PCOS and physical symptoms may be more likely to present to an endocrinological clinic, compared with females with PCOS and biochemical abnormalities (including high Free Testosterone levels) but without physical symptoms, the syndrome may go undetected in this population thereby excluding a large group of females with PCOS, which may affect overall results. Additionally, results would apply only to this subsection of the population at large which may not be a representative group. This may, in turn, obscure results to some extent. However, the question still remains whether symptoms of low mood are a direct consequence of biochemical abnormalities such as increased testosterone levels, or are present due to the physical symptoms of PCOS. Some studies have found no association between the physical symptoms of PCOS and mood or anxiety (Barth et al., 1993; Hollinrake et al., 2007; Karjula et al., 2017; Shulman et al., 1992). Shulman et al. (1992) found an increased incidence of depression in facially hirsute females which correlated with serum levels of Free Testosterone but not with the extent of facial hirsutism.

5.3.2.5.2 Correlational versus categorical analyses
Overall, studies including both PCOS and non-PCOS samples used two main methods to determine the relationship between androgens and symptoms of depression. The first was a correlation between levels of depression and levels of key androgens, which was subject to difficulties related to the type of scale used. Second, some studies split the PCOS group into those with depression and those without (Annagür et al., 2013; Bazarganipour et al., 2013; Bhattacharya & Jha, 2010; Hollinrake et al., 2007; Jedel et al., 2011; Klimczak et al., 2015; Rahiminejad et al., 2014; Rocco et al., 1991), or into groups within the diagnosis of PCOS based on specific criteria (NIH and non-NIH) (Moran et al., 2012), and then examined between-group differences in testosterone levels. In this situation, in most cases, a scale with a predetermined cut-off to diagnose depression was used, which may not be as useful as a clinical interview (see next section). Regardless of the method of determining the relationship and the scales used, the data does not strongly support a direct relationship between testosterone and depression.

5.3.2.5.3 Depression rating scales

Depression rating scales, as opposed to diagnostic interviews, measure differences in severity of depression symptoms, or changes in depressive symptoms over time. There are, however, many different depression rating scales that serve different purposes. Inconsistencies in findings may be explained, in part, by the use of particular depression rating scales. Rating scales are usually designed to either: 1) measure change in depression during treatment trials, in which case they are designed for people with significant clinical depression and may not detect correlations at lower levels of depressive symptoms, such as those found in PCOS (mild-moderate severity), 3) scales that are used to measure low levels of mood variability in normal populations, for example, the POMS 2) screen for depression and are, therefore, developed in order to have a cut-off but not to be sensitive to differences in levels of depression at a higher severity. With regards to PCOS samples, the preference would be to use depression rating scales
that detect low levels of depression symptoms, however, most studies examining mood in PCOS samples have used scales that are designed to assess more severe depressive symptoms. The former is of particular relevance, as despite findings of more depression and anxiety in females with PCOS, the severity of depression in PCOS remains to be that of moderate severity (Barry, Kuczmierczyk, et al., 2011), and therefore, there needs to be a depression measure with appropriate sensitivity to pick up associations relevant to lower symptoms of depression.

Additionally, many studies used self-report questionnaires such as the HADS, BDI, and QIDS (Barry, Kuczmierczyk, et al., 2011; Bazarganipour et al., 2013; Hollinrake et al., 2007; Livadas et al., 2011; Moran et al., 2012; Pastore et al., 2011; Rasgon et al., 2003). The most commonly used depression rating scale to assess mood in PCOS samples was the BDI (Adali et al., 2008; Cinar et al., 2011; Hollinrake et al., 2007; Klimczak et al., 2015; Livadas et al., 2011). Overall, in the current review, studies that reported significant positive associations tended to measure Free Testosterone or FAI levels. These studies finding an association between FAI levels and mood tended to be larger compared with studies which found associations in contrasting directions and studies which found non-significant relationships. Additionally, the three studies that found a positive association used the HADS including Cinar et al’s study (2011) which included the BDI together with the HADS to assess symptoms of depression. As noted earlier (section 5.3.1.4), the HADS was developed to examine symptoms of anxiety and depression in medical populations (for example, females with PCOS). It does not focus on the somatic symptoms of depression, such as loss of energy, sleeping patterns, appetite and tiredness or fatigue, which could be related to the medical condition experienced by an individual rather than a reflection of low mood. The BDI, however, includes somatic symptoms of depression, and therefore, is a more suitable instrument to use in psychiatric populations whereas the HADS may be better suited for medical populations such as PCOS, providing a more accurate estimate of symptoms of depression in these populations and may operate at the correct part of the spectrum of symptoms of depression.
Other measures used to assess depression in the reviewed studies were not designed specifically to measure current severity or levels of depression and may be less sensitive to subtle differences in mood state, therefore, less likely to show correlations with factors which impact mood states such as testosterone levels, for example, the Minnesota Multiphasic Personality Inventory (Rocco et al., 1991), the Mini International Neuropsychiatric Interview (MINI) (Mansson et al., 2008), the Neuroticism-Extraversion-Openness Personality Inventory (NEO-PI-R), the Affect Balance Scale, the Multiple Affect Adjective Check List, Health-Related Quality of Life, and the Symptom Checklist Survey (Derogatis et al., 1993; Hahn et al., 2005; S. Phillips & Sherwin, 1992; Rocco et al., 1991; Schutter et al., 2017; Shulman et al., 1992). Only two small studies (n = 58) used a clinician-administered measure of depression rather than a self-report depression rating scale to determine presence or absence of clinically significant depression and found a significant association between androgen levels and depression and anxiety in patients with PCOS, however, in opposite directions. Annagur et al. (2013) in a small study found significantly higher androgen (DHEAS) levels in females with PCOS and MDD (n = 23) (Structured Clinical Interview for DSM-IV; SCID-I) compared with females with PCOS without MDD (n = 30) (p = 0.001), whereas Jedel et al. (2011) (n = 35) found lower androgen levels) (Total and Free T levels, p = 002) in females with PCOS with higher symptoms of depression (MADRS) compared with females with PCOS with lower symptoms (n = 37). However, these studies measured different forms of androgens, are very small and potentially underpowered to show significant results.

5.3.3 Androgen levels and cognitive function in observational studies

Nineteen studies with an observational design assessed the relationship between androgen levels and cognitive function in reproductive-aged females.
5.3.3.1 Verbal learning and memory

Seven cross-sectional studies measured verbal learning and memory in relation to androgen levels (Gómez-Gil et al., 2009; Halari et al., 2005; Hussain, Hanafi, Konishi, Brake, & Bohbot, 2016; Janowsky, Chavez, Zamboni, & Orwoll, 1998; S. Phillips & Sherwin, 1992; Romero-Martínez, González-Bono, Salvador, & Moya-Albiol, 2015; Schattmann & Sherwin, 2007b). Five of seven studies found no significant relationship ($n = 178$) while the remaining two ($n = 49$) reported inconsistent findings (S. Phillips & Sherwin, 1992; Romero-Martínez et al., 2015). In a sample of 25 healthy females (S. Phillips & Sherwin, 1992), lower Free Testosterone levels were associated with better verbal memory performance (Wechsler Memory Scale). Romero-Martínez et al. (2015) found a curvilinear relationship between Free Testosterone and verbal memory in a sample of 24 mothers of children with autism spectrum disorder on an isolated aspect of the RAVLT, with no specific correlation between Free Testosterone and verbal memory found in the control group with mothers of healthy children ($n = 22$).

Overall, these results suggest minimal evidence of a relationship between androgen levels and verbal learning and memory.

5.3.3.2 Verbal ability

Three studies assessed verbal ability (Gouchie & Kimura, 1991; Halari et al., 2005; McKeever, Rich, Deyo, & Conner, 1987) (Tongue Twister Task, Vocabulary Sub-test of WAIS-R, Shiplet Hartford Vocabulary Test) and found no significant correlations between verbal ability and androgen levels.

5.3.3.3 Visuospatial learning and memory

There was no significant association between androgen levels and spatial learning and memory in three of four studies (Halari et al., 2005; Janowsky et al., 1998; Schattmann & Sherwin, 2007b). The remaining small study found significantly better visual memory performance...
(Visual Paired Associates) in a testosterone-treated group \((n = 9)\) compared with the no-treatment group \((n = 10)\) in 33 female-to-male transsexuals (Gómez-Gil et al., 2009). There were no significant differences in verbal memory between groups in this study.

5.3.3.4 Visuospatial ability

Six of 16 studies \((n = 170)\) found a significant relationship between spatial ability and androgen levels (Barry et al., 2013; Burkitt, Widman, & Saucier, 2007; Gouchie & Kimura, 1991; Hausmann et al., 2000; Moffat & Hampson, 1996; Shute, Pellegrino, Hubert, & Reynolds, 1983). Moffat et al. (1996) found a significant positive correlation between spatial ability (Mental Rotations Task [MRT] and Paper Folding Test) and Free Testosterone levels, however, this finding was limited to right-handed females only \((n = 19)\). Gouchie and Kimura (1991) did not find a significant correlation between Free Testosterone levels and spatial ability (Paper Folding) but found significantly better performance in a high testosterone group compared with low testosterone group \((p < 0.03)\) \((n = 46)\) on this task. Burkitt et al. (2007) found significantly worse spatial ability in females with low androgen levels \((n = 18)\) compared with females with high androgen levels \((n = 19)\) on an aspect of a Virtual Water Maze Task (females with low testosterone levels needed significantly longer time to complete the task \((M = 96.769 \pm 66.395 SD)\) compared with females with higher testosterone levels \((M = 47.481 \pm 41.023 SD, p < 0.05)\).

In a small study \((n = 12)\), females were divided into high and low testosterone groups \((n = 6\) in each group), and females with higher Free Testosterone levels performed significantly better on a Spatial Visualisation Task compared with females with lower Free Testosterone levels (Shute et al., 1983). However, in the first part of the same study (Shute et al., 1983), correlational analyses showed no significant association between Free Testosterone levels and spatial ability \((n = 48\) healthy females). A small longitudinal, observational study \((n = 12)\) found a significant positive correlation between testosterone (unspecified) levels and spatial ability (Vandenberg and Kuse’s MRT) in healthy females (Hausmann et al., 2000). In a PCOS
sample ($n = 69$), Barry et al. (2013) found a significant positive correlation between Total Testosterone levels and spatial ability (3-D MRT) ($r = 0.376$, $n = 56$, $p < 0.002$) compared with 41 sub-fertile healthy females.

The 10 remaining studies ($n = 394$), including one with females with PCOS (Schattmann & Sherwin, 2007b), found no significant association between androgen levels and visuospatial ability (Stafford Identical Blocks Test, Minnesota Paper Form Board Test, Spatial Relations Test) (Durdiaková, Hodosy, Kubranská, Ostatniková, & Celec, 2012; Falter, Arroyo, & Davis, 2006; Halari et al., 2005; Hassler, Gupta, & Wollmann, 1992; Hussain et al., 2016; Janowsky et al., 1998; McKeever et al., 1987; Neave, Menaged, & Weightman, 1999; Puts et al., 2010).

5.3.3.5 Psychomotor speed

Two of three studies including one with a PCOS sample examining psychomotor speed found a significant relationship between this domain and androgen levels. FAI levels significantly negatively correlated with motor speed in a sample of 28 females with PCOS (higher FAI levels associated with slower performance on the Purdue Pegboard Task) (Schattmann & Sherwin, 2007b). In contrast, another study found psychomotor speed (Dart-Throwing Task) to be significantly positively correlated with Free Testosterone levels (higher Free Testosterone levels associated with faster performance) (Janowsky et al., 1998). In this study, the significant correlation was found in females using their dominant hand, however, a significant negative correlation was found in females using their non-dominant hand (total $n = 30$). The finding is, therefore, somewhat mixed. One study found no significant association between psychomotor speed (Finding A’s and Identical Pictures Test) and Free Testosterone levels ($n = 46$) (Gouchie & Kimura, 1991).
5.3.3.6 Executive function

No evidence was found regarding a relationship between executive function and androgen levels in five studies including healthy females (total $n = 149$) (Halari et al., 2005; Hassler et al., 1992; McKeever et al., 1987; Moffat & Hampson, 1996; Neave et al., 1999) and one with 28 females with PCOS (Schattmann & Sherwin, 2007b).

5.3.3.7 Summary of findings

Overall, no strong evidence of a relationship between androgen levels and verbal learning and memory or verbal ability was found in five observational studies. Only two studies found significant yet mixed results with one associating lower Free Testosterone levels with better verbal memory (S. Phillips & Sherwin, 1992) while the other finding a curvilinear relationship between Free Testosterone levels and verbal memory (one aspect of the RAVLT) (Romero-Martínez et al., 2015). No strong evidence regarding the relationship between androgen levels and attention and executive function was found. Only one small study out of four studies examining visuospatial memory in relation to testosterone levels found better visual memory in a testosterone-treated group (Gómez-Gil et al., 2009). There was mixed evidence regarding the association between Free Testosterone/FAI levels and psychomotor speed with three studies examining this relationship and finding a positive (Janowsky et al., 1998), negative (Schattmann & Sherwin, 2007b) and no association (Gouchie & Kimura, 1991).

Until now, a well-studied cognitive domain in relation to testosterone levels seems to be visuospatial ability. Although 10 studies ($n = 394$) found no significant association between spatial ability and androgen levels, six studies found a significant and consistent positive relationship ($n = 170$). Females with relatively higher levels of testosterone were found to show better performance on spatial ability tasks compared with females with lower testosterone levels. Studies have shown gender differences in spatial abilities with males outperforming females (Choi & Silverman, 2002; Halpern, 2000; Linn & Petersen, 1985; Maccoby & Jacklin,
1978; Masters & Sanders, 1993), which may, however, relate to the organisational effect of testosterone. Findings related to activational effects of testosterone, i.e., change in visuospatial ability following testosterone administration will be discussed in the following section.

5.3.4 Androgen levels and cognitive functioning in interventional/experimental studies

Nine studies with an interventional study design investigated the relationship between androgen levels and cognitive function in females of reproductive age. Only one study included patients with PCOS (Schattmann & Sherwin, 2007a).

5.3.4.1 Verbal learning and memory

Only one study measured verbal learning and memory and found no significant changes following anti-androgen treatment in females with PCOS ($n = 8$) on the RAVLT, Logical Memory Test, and the Paired Associates Test (Schattmann & Sherwin, 2007a).

5.3.4.2 Verbal ability

Verbal ability was measured using the Verbal Reasoning Test in four studies (Slabbekoorn, Van Goozen, Megens, Gooren, & Cohen-Kettenis, 1999; Van Goozen, Cohen-Kettenis, Gooren, Frijda, & Van de Poll, 1994, 1995; Van Goozen, Slabbekoorn, Gooren, Sanders, & Cohen-Kettenis, 2002) including transsexual participants, and the Vocabulary sub-test of Wechsler Adult Intelligence Scale-III in one study including participants with PCOS (Schattmann & Sherwin, 2007a). None of the five studies involving testosterone or anti-androgen administration in transsexual or PCOS participants found a significant association between testosterone and verbal ability.
5.3.4.3 Visuospatial learning and memory

Two interventional studies included a measure of visuospatial learning and memory (Pintzka, Evensmoen, Lehn, & Haberg, 2016; Postma et al., 2000). On the Object-Location Memory Task, Postma et al. (2000) found no effect of testosterone administration on the first two task conditions (Positional Reconstruction and Object-to-Position Assignment) but found better performance on the third (combined) condition (assessing immediate and delayed recall) following testosterone administration \((n = 8)\), compared with the placebo condition \((n = 7)\) in healthy females. Pintzka et al. (2016) found better visuospatial learning (representation of direction on the Virtual Environment Learning and Navigating Task) in the testosterone group \((n = 21)\) compared with the placebo group \((n = 21)\) (corrected \(p < 0.02)\).

5.3.4.4 Visuospatial ability

Significant improvement in spatial ability was found in six of eight studies following testosterone administration (Aleman, Bronk, Kessels, Koppeschaar, & van Honk, 2004; K. Miller, Grieco, & Klibanski, 2005; Pintzka et al., 2016; Slabbekoorn et al., 1999; Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995). Two studies found significant improvement in visuospatial ability (Rotated Figures Test, Card Rotation Task) in female-to-male transsexual samples (total \(n = 57\)) following testosterone administration over a period of three months (Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995). Slabbekoorn et al. (1999) found a significant improvement in spatial ability scores (Rotated Figures 2 and 3-Dimensional and Hidden Figures Task) following testosterone administration over 1.5 years in a sample of 20 female-to-male transsexuals. Similarly, Miller et al. (2005) found baseline Free Testosterone levels to be significantly positively correlated with spatial ability (3-D Mental Rotation score) in a clinical sample of 33 females with anorexia. Testosterone administration \((n = 24)\) for three weeks was followed by significant improvement in visuospatial ability compared with the placebo condition \((n = 9)\).
In single-dose administration studies, Aleman et al. (2004) found a significant improvement in spatial ability (Mental Rotation score) in 26 healthy females following testosterone administration ($n = 14$) compared with a placebo condition ($n = 12$). Similarly, Pintzka et al. (2016) found that a single dose of testosterone in healthy females ($n = 21$) was associated with significantly better performance on one spatial ability measure (Mental Rotations Test) compared with placebo condition ($n = 21$); however, in this study, no differences were found in the other measure of spatial ability (fMRI stimuli with a self-paced Block Design Task).

Two studies found no significant change in visuospatial ability following testosterone administration and anti-androgen treatment, respectively, in females with and without PCOS (Schattmann & Sherwin, 2007a; Van Goozen et al., 2002). In the Van Goozen et al. study (2002) no effect of testosterone treatment over a span of 14 weeks was observed on spatial ability (Line Orientation Test, Rotated Figures (2-D and 3-D), Targeted Throwing Task) in female-to-male transsexuals ($n = 19$) in comparison with healthy females who did not receive treatment over the course of 14 weeks ($n = 22$). This result may be explained by the fact that the male and female brain are fundamentally different in many ways and may react differently to testosterone. Therefore, an increase in testosterone levels in a female brain (involving testosterone treatment) does not necessarily change its function to a more male pattern. Given that the administration of testosterone induces large changes in testosterone levels, this may be more likely to show a change in cognitive function but may have less relevance to the usual physiology of the female/male brain. It is important to note, however, that while some studies suggest that sex differences in the brain are significantly widespread (Cahill, 2006), other studies postulate that these findings are not entirely accurate (Eliot, 2011; Rippon, Jordan-Young, Kaiser, & Fine, 2014), reviewed by McCarthy (2016). The other study including PCOS samples involved anti-androgen treatment (CPA) over a course of three months and found no significant changes in spatial ability (MRT, Paper Folding, Water Level Test) post-treatment (Schattmann & Sherwin, 2007a). However, in this sample of 19 hirsute PCOS females, post-
treatment FAI levels were significantly negatively correlated with spatial visualisation scores (Paper Folding), suggesting that lower levels of FAI were associated with better spatial performance, which is in contrast to findings from other studies.

Overall, results suggest a positive association between testosterone and visuospatial ability with higher levels of testosterone related to improvements in visuospatial ability.

5.3.4.5 Psychomotor Speed

Two treatment studies measured psychomotor speed, however, neither found a significant effect of administration of testosterone on this domain (Schattmann & Sherwin, 2007a; Slabbekoorn et al., 1999).

5.3.4.6 Executive function

Executive function (verbal fluency) was assessed by four interventional studies involving both androgen and anti-androgen treatment. In 19 hirsute females (Schattmann & Sherwin, 2007a), verbal fluency (COWAT) significantly improved following anti-androgen treatment \((n = 8)\) compared with placebo \((n = 11)\). However, post-treatment androgen levels (FAI) were not significantly correlated with improved verbal fluency. Reduced verbal fluency (Word and Sentence Production) was found following androgen treatment in both longitudinal studies involving female-to-male transsexual samples (Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995). Another study found no significant change in verbal fluency (Word and Sentence Task) following testosterone treatment in a sample of 25 female-to-male transsexuals (Slabbekoorn et al., 1999). Overall, results tend to indicate that androgen treatment results in worse performance on measures of verbal fluency, while anti-androgen treatment improved performance.
5.3.4.7 Summary of findings

Previous research has focused on the organisational effects of testosterone involving an examination of gender differences in specific cognitive abilities. Verbal fluency is recognised to be a female-superior cognitive domain (Halpern, 2000) while males generally have an advantage in visuospatial function (Choi & Silverman, 2002; Linn & Petersen, 1985; Maccoby & Jacklin, 1978; Masters & Sanders, 1993). In the current review, although no significant findings related to organisational effects of testosterone were obtained from cross-sectional studies regarding the relationship between androgen levels and verbal fluency, interventional studies found androgen treatment to be associated with decreased verbal fluency performance (Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995) whereas anti-androgen treatment appeared to benefit verbal fluency (Schattmann & Sherwin, 2007a). Since females with PCOS have elevated testosterone levels, it would be expected that they may show an advantage over healthy females on tasks assessing spatial ability, which also have a significant male-advantage. However, the current review found no significant association between testosterone levels and spatial ability in females with PCOS (Barry et al., 2013; Schattmann & Sherwin, 2007b). Some studies including both cross-sectional and double-blind placebo controlled trials found improved performance on tasks assessing spatial visualisation and mental rotation ability following testosterone administration compared with the placebo condition in healthy females (Aleman et al., 2004; Burkitt et al., 2007; Gouchie & Kimura, 1991; Hausmann et al., 2000; K. Miller et al., 2005; Moffat & Hampson, 1996; Pintzka et al., 2016; Shute et al., 1983; Slabbekoorn et al., 1999; Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995), however, many other studies examining this association did not find significant results related to this domain (Durdiaková et al., 2012; Falter et al., 2006; Halari et al., 2005; Hassler et al., 1992; Hussain et al., 2016; Janowsky et al., 1998; McKeever et al., 1987; Neave et al., 1999; Puts et al., 2010). These findings are, of course, different from those in PCOS samples with naturally and abnormally elevated
testosterone levels. Overall, in PCOS samples, there seems to be no significant association between testosterone levels and spatial ability.

5.4 ANDROGEN LEVELS AND EMOTION PROCESSING

The relationship between androgen levels and emotion processing was examined by two cross-sectional studies and ten interventional studies.

5.4.1 Cross-sectional studies

Two small cross-sectional studies (Stanton, Wirth, Waugh, & Schultheiss, 2009; van Honk et al., 1999) investigated the association between androgen levels and emotion processing in females, with one finding a relationship between attending away from angry faces and higher testosterone levels, while the other found no significant association. In 16 healthy females, higher Free Testosterone levels were significantly associated with attending away from angry faces (Emotional Stroop Task - Pictures of Facial Affect) (van Honk et al., 1999). However, this finding was limited only to testosterone samples taken 6 hours prior to testing (reflecting the highest levels of testosterone), testosterone samples collected at other times were not significantly correlated, which may be a chance finding due to multiple testing. Stanton et al. (2009) found no significant correlation (regression analysis) between emotion processing (Face Stimulus Test) and Free Testosterone levels \((n = 14)\). Additionally, no significant correlation was found between brain activity (amygdala and ventromedial prefrontal cortex) and Free Testosterone levels during the emotion processing task in this study.

5.4.2 Interventional studies

Ten interventional studies assessed varied aspects of emotion processing in relation to testosterone levels (Bos et al., 2016; Bos et al., 2013; Enter, Terburg, Harrewijn, Spinhoven, & Roelofs, 2015; Hermans et al., 2007; Olsson et al., 2016; Terburg, Aarts, & van Honk, 2012; van Honk et al., 2005; van Honk & Schutter, 2007; van Honk et al., 2011; van Wingen et al.,
Four of ten studies examining the relationship between testosterone and facial emotion processing suggested that testosterone administration reduced aspects of the response to threatening facial stimuli, including faces expressing fear or anger (Hermans et al., 2007; Terburg et al., 2012; van Honk et al., 2005; van Honk & Schutter, 2007). van Honk et al. (2005), in a sample of 16 healthy females, found that the vigilant emotional response to masked fearful faces (Masked Emotional Stroop Task) observed in the placebo condition significantly reduced following testosterone administration. In another study, testosterone administration was followed by a reduced sensitivity in recognising angry faces compared with the placebo condition in 16 healthy females (van Honk & Schutter, 2007). However, in this study, no effect of testosterone was observed for faces expressing fear or disgust. Similarly, a significant delay in gaze aversion (gaze or attendance away from/endurance of attendance to threatening faces) from masked angry faces was found in another study including 20 healthy females following testosterone administration compared with a placebo condition (Social Dominance Task) (Terburg et al., 2012). Greater tolerability or a greater delay in gaze aversion further indicate reduced sensitivity toward threatening angry faces. In the final study, Hermans et al. (2007) found a reduced anxiety response (lower skin conductance and reduced affective startle response) to negative-valence pictures in 20 high trait-anxiety participants (STAI) following testosterone administration.

In a test of “social intelligence”, van Honk et al. (2011) found a significant decrease in the score on the Reading the Mind in the Eyes Test (RMET) following testosterone administration compared with the placebo condition ($n = 16$). In the same study, a significant negative correlation was found between endogenous Free Testosterone levels and RMET scores in the placebo condition. Another randomised double-blind study (Olsson et al., 2016) showed similar results with significantly reduced scores on the RMET following testosterone administration. Female participants ($n = 33$) performed less accurately on the task compared with the placebo condition (Olsson et al., 2016). In these studies, however, no significant effect of testosterone
administration was found on self-reported anxiety and/or mood (STAI, POMS) (Olsson et al., 2016; van Honk et al., 2011). However, as discussed earlier, the POMS may not be an accurate reflection of symptoms of low mood as found in depression. To summarise, results indicate that increasing testosterone levels in females may be related to a decreased vigilant emotional and startle response, delay in gaze aversion, and decreased facial emotion processing which may all relate to social anxiety, and also to aspects of reduced “social intelligence”.

Four brain-imaging studies found a significant association between brain activity during emotion processing tasks and testosterone administration (Bos et al., 2016; Bos et al., 2013; Enter et al., 2015; van Wingen et al., 2009), however, only one found a significant direct effect of testosterone administration on performance on the emotion processing task (Enter et al., 2015). In a sample of 18 females with Social Anxiety Disorder (SAD), a significant decrease in gaze avoidance for angry faces was observed following testosterone administration (Enter et al., 2015). Gaze avoidance is understood to be a characteristic feature of SAD (Moukheiber et al., 2010; M. Stein & Stein, 2008) and individuals with SAD have been generally found to show low levels of testosterone compared with healthy individuals (Giltay et al., 2012). Studies have also demonstrated avoidance of the eye-region of angry faces in individuals with SAD (Horley, Williams, Gonsalvez, & Gordon, 2004). Enter et al.’s (2015) study showed a significant effect of a single administration of testosterone on gaze avoidance toward angry faces (reduced gaze avoidance) in females with SAD ($n = 18$) but not in healthy controls ($n = 19$). However, no significant change was found in performance on the task assessing facial emotion processing (NimStim task involving angry, happy and neutral facial expressions) following testosterone administration in this study. Three remaining studies ($n = 53$) found significant changes in brain activity in areas crucial for emotion processing (including increased amygdala activity, reduced connectivity between left inferior frontal gyrus, anterior cingulate cortex and supplementary motor area, reduced neural response to emotional faces in the precuneus) following testosterone administration compared with the placebo condition (Bos et al., 2016; Bos et al., 2013; van
Wingen et al., 2009). However, no direct effect of testosterone administration on measures of emotion processing (emotion condition of the Blocked Design Task, Dynamic Facial Expression Task, adaptation of the RMET) was detected in any of the studies (Bos et al., 2016; Bos et al., 2013; van Wingen et al., 2009). In addition, androgen levels were not found to be significantly correlated with self-reported mood (Mood Rating Scale and POMS) or anxiety (STAI) in these studies.

Overall, these results suggest that testosterone administration reduces sensitivity of response to threatening facial stimuli, particularly faces expressing anger and fear, and reduces anxiety response by altering physiological skin and affective startle response (Hermans et al., 2007; Terburg et al., 2012; van Honk et al., 2005; van Honk & Schutter, 2007). Testosterone administration was found to be associated with decreased social anxiety (Enter et al., 2015; Terburg et al., 2012) observed through reduced gaze avoidance (and increased fixation toward the eye region), reduced physiological anxiety response, and attentional bias, which seems to be a consistent finding. Data suggests that testosterone administration reduces attention towards threat stimuli while reducing gaze avoidance (a socially anxious pattern) and reduces startle response and skin conductance, both in healthy volunteers, and in high trait anxiety and social anxiety (Hermans et al., 2007).

5.4.3 Overall summary

Observational studies were less likely to find significant positive results, perhaps due to issues of relatively compressed ranges of testosterone levels, normal fluctuation in androgen levels, and heterogeneous groups. Studies tended to find worse performance on tests assessing “social intelligence” (RMET) following testosterone administration (Olsson et al., 2016; van Honk et al., 2011). Van Honk et al. (2011) also found a significant negative correlation between baseline Free Testosterone levels and scores on a computerised adaptation of the RMET (higher testosterone levels associated with worse empathy). This is consistent with findings showing
testosterone’s capacity to alter the processing of facial threat, particularly facial indicators of fear and anger which promote sociality through an empathic understanding of other people’s emotions. (Baron-Cohen, 2002; J. Harris, Rushton, Hampson, & Jackson, 1996; Hermans et al., 2006; van Honk & Schutter, 2007). Studies have shown a negative association between empathy, crucial for understanding or inferring the emotional states of others and important for healthy social functioning, and testosterone levels (Olsson et al., 2016; van Honk et al., 2011).

There was strong evidence for an association between emotion processing and changes in brain activity following testosterone administration in neuroimaging studies (Bos et al., 2016; Bos et al., 2013; Enter et al., 2015; van Wingen et al., 2009). Although data consistently shows reduced sensitivity toward fearful and threatening emotional faces (observed through reduced gaze aversion and avoidance and reduced recognition accuracy of dominant angry faces) neuroimaging studies involving testosterone administration in humans have consistently shown increased amygdala reactivity (Bos et al., 2013; van Wingen et al., 2009). The amygdala is strongly linked to neural networks involved in fear responses and is sensitive to emotional facial expressions (Phelps & LeDoux, 2005). Patients with mood and anxiety disorders have been found to show exaggerated amygdalar responses to emotional faces compared with controls (Bos et al., 2013; Drevets, 2003; Monk et al., 2008). Additionally, androgen receptors are present in the amygdalar region and so this region is, therefore, a crucial target for testosterone (Sarkey, Azcoitia, Garcia-Segura, Garcia-Ovejero, & DonCarlos, 2008). However, one study found that testosterone administration appears to selectively increase activation of the superficial amygdala and to a lesser extent the basolateral amygdala, and an increased activation of these sub regions of the amygdala is consistent with a fear-reducing effect of testosterone (Bos et al., 2013). One brain imaging study found a significant reduction in connectivity between the left inferior frontal gyrus (IFG) and the anterior cingulate cortex (ACC) and supplementary motor area (SMA) crucial for the emotion processing task (RMET) compared with the placebo condition (Bos et al., 2016). Results suggest that these brain regions are crucial
for optimal performance on the emotion processing test. The above mentioned brain regions are involved in integrating sensory information and for planning behaviour during emotion processing. Studies have demonstrated worse performance following testosterone administration on the RMET which involves inferring emotions or mental states based on pictures of the eye-region (Olsson et al., 2016; van Honk et al., 2011). However, studies investigating changes in brain activity in relation to testosterone did not find a direct association between testosterone administration and performance on emotion processing measures.

5.5 CONCLUSION

This chapter investigated the relationship between androgen levels and symptoms of depression and anxiety, cognitive function, and emotion processing performance in females of reproductive age. The main reasons for conducting this systematic review were:

1) There is a clear link between PCOS, characterised by androgen excess, and depression. The relationship of low mood to high levels of androgens in this condition is not, however, as clear (Cooney et al., 2017) and neither is the effect of anti-androgen treatment on depression. Additionally, there is limited evidence related to cognitive function and emotion processing in females with PCOS. It is, therefore, useful to examine the evidence regarding the direct association between androgen levels, and mood, cognitive and emotion processing performance in females with PCOS.

2) Literature suggests that testosterone levels are positively correlated with male-favouring cognitive domains such as visuospatial ability and visuospatial learning and memory and negatively correlated with female-favouring cognitive domains including verbal learning and memory, psychomotor speed and verbal fluency (Halpern, 2000). However, this association is not clearly understood in females of reproductive age, particularly in samples
with PCOS showing abnormally elevated levels of Free Testosterone and FAI levels. Therefore, one of the aims of the current review was to investigate whether there was an association between androgen levels and cognitive function and emotion processing in females of reproductive age with and without PCOS.

3) Until now only one small neuroimaging study has evaluated emotion processing in females with PCOS, however, the primary outcome of interest in this study was neuronal activation using fMRI during an emotion processing task. The aim of the current review was to examine testosterone levels in relation to emotion processing in both observational and experimental studies (involving testosterone administration). Experimental studies suggest that testosterone administration reduced sensitivity of response demonstrated through reduced gaze aversion and gaze avoidance, decreased physiological startle response, and decreased selective attention to threatening (anger and fear) faces following testosterone administration. Decreased social cognition (empathic response) was observed following testosterone administration.

4) Studies that found a positive association between greater depressive symptomatology and higher levels of Free Testosterone and/or FAI used the HADS as the primary depression rating scale compared with studies that did not find any significant associations.

5) Regarding cognitive function, no significant association was found between executive function (verbal fluency) in cross-sectional studies but interventional studies found androgen treatment to be associated with decreased verbal fluency performance whereas anti-androgen treatment appeared to benefit verbal fluency. No significant association was found between testosterone levels and spatial ability in females with PCOS. There was mixed evidence regarding the relationship between testosterone levels and visuospatial ability in interventional studies. Overall the relationship between cognitive function and testosterone levels needs further study.
6.1 INTRODUCTION

The methods and materials for the current study will be described in this chapter. The rationale for the selection of tests used will also be provided.

6.2 MAIN STUDY DESIGN

The study was approved by the University of Otago Human Ethics Committee (Health) (see Appendix E). Participants gave written informed consent prior to study inclusion (see Appendices A and B for information sheets and consent forms).

The study was a longitudinal, parallel-group study. Testing sessions were conducted at two time-points (baseline and 12 weeks after) for both patient and control groups. At each time-point, measures of mood, cognitive functioning, emotion processing, and physical symptoms were collected. The study was naturalistic, in that the nature and choice of treatment for the patient group was the responsibility of the endocrinologist who worked at Christchurch Women’s Hospital and at a private clinic. All included patients had abnormalities in the androgen system and were treated with standard hormonal medication. The dosage/type of medicine depended on a variety of clinical factors. Non-PCOS control participants completed the same assessments, but did not receive treatment.
6.3 PARTICIPANTS

6.3.1 PCOS patients

There were two pathways for recruitment of the clinical sample:

1) females referred to gynaecological endocrine clinics at Christchurch Women’s Hospital (public hospital) and the Women’s Health Clinic at Southern Cross Hospital (private hospital) in Christchurch, or,

2) females responding to advertisements in local newspapers (The Christchurch Star Newspaper and The Metropol) seeking volunteers with untreated symptoms of PCOS.

Regardless of the pathway for recruitment, all patients were treated by the same Gynaecological-Endocrinologist, who assessed their hormonal profile and put a treatment plan in place depending on their condition.

Inclusion criteria were as follows:

1. females aged between 16 and 40 years,
2. diagnosis of PCOS by the Gynaecological-Endocrinologist
3. pre-menopausal, and
4. able to provide informed consent.

Reasons for exclusion included:

1. taking exogenous hormones prior to the study,
2. neurological conditions (e.g., multiple sclerosis), major psychiatric conditions (e.g., bipolar disorder or schizophrenia), or major chronic medical illnesses (e.g., cancer or HIV),
3. current serious alcohol and/or substance dependence,
4. menopause or hysterectomy,
5. pregnancy,
6. previous head injury resulting in loss of consciousness for more than an hour,
7. other endocrinological abnormality apart from excess androgen levels,
8. infertility treatment (the current study involved androgen-lowering medication, including agents such as oral contraceptive pills, which are considered appropriate first-line treatment in females with no desire to conceive),
9. insufficient visual or auditory functioning for completion of cognitive tests, and
10. non-fluency in English.

Once patients expressed interest in participation, they were provided with an information sheet (see Appendix A) and were then contacted by the PhD student to discuss further details of study participation and to complete preliminary screening. Opportunity was given to ask questions, and to call friends or family/whānau for advice or support, before deciding whether to consent to taking part in the study. After providing consent (see Appendix B for the consent form), baseline assessment was organised and completed before treatment began. Baseline assessment involved a more thorough screening of major neurological, psychiatric, or medical conditions.

6.3.2 Control participants (Non-Polycystic Ovarian Syndrome)

Control participants were eligible for inclusion provided they were pre-menopausal females aged between 16 and 40 years and showed normal androgen levels along with no physical symptoms of PCOS, such as hirsutism, severe acne, or ovarian cysts. Control participants were recruited over the same period as patients, using flyers posted in public places around Christchurch, including shopping malls, health centres, gymnasiums, university campuses (see Appendix C), and online resources (http://www.subjectswanted.co.nz/). Once a potential control participant had expressed interest, the study information sheet was mailed to them and any questions were answered over email or phone. Screening questions were conducted over the phone, before recruitment and assessment began. The control group had the same exclusion criteria as patients. Please see a flowchart (Figure 6.6) for an overview of study design, which
describes the relationship between Study 1 (baseline) and Study 2 (following treatment), and the nature of the two groups involved in these studies.

6.3.3 Matching of control participants to PCOS patients

Patients and control participants were matched in a pair-wise manner for age (within five years of age). An attempt was made to match the two groups for estimated premorbid IQ at a group level by advertising in places in which a representative sample of the population would be present, including malls, gyms, universities and libraries.

The method of obtaining menstrual status and premorbid IQ is described below.

6.3.3.1 Menstrual status

Only reproductive-aged/pre-menopausal females were included in the current study. Control participants were classified as being in either the follicular or the luteal phase of their menstrual cycle, determined by recording the date the last menstrual period started, and the usual length of the menstrual cycle. The follicular phase (and consequently the menstrual cycle) varies widely, but the luteal phase is a known number of days. Control participants were typically tested at the same phase of their menstrual cycle at baseline and follow-up testing. Since females with PCOS often have highly irregular menstrual cycles or experience amenorrhea, this degree of consistency in menstrual cycle phases between baseline and follow-up was not possible to record in this group.

6.3.3.2 Estimated Premorbid IQ

Premorbid verbal IQ was estimated using the National Adult Reading Test (NART) (Nelson & Willison, 1991). It comprises of 50 words printed in order of increasing difficulty and is considered to be a reliable measure of premorbid verbal IQ in clinical use (Spren & Strauss, 1998). The value of the test lies in its ability to produce an accurate estimate of premorbid IQ.
through word reading, since word reading tends to be preserved in abnormal aging and other neurological disorders (Crawford, Deary, Starr, & Whalley, 2001). Phonetically irregular and relatively short words are used (e.g., ‘ache’, ‘naïve’, ‘thyme’), as well as generally unfamiliar words (e.g., ‘assignate’, ‘demense’, ‘campanile’). Reading of an unfamiliar word depends on the individual’s ability to phonetically decode the constitutional elements of the word rather than reliance on prior familiarity with the word (Nelson & Willison, 1991). Participants read the list of words aloud and the number of errors were recorded by the interviewer.

The NART produces a predicted verbal IQ score, as calculated by the total number of mispronunciation errors. United Kingdom norms for the NART were used in this study, due to limited availability of New Zealand norms at the time of study development. Correct pronunciation of NART words was determined from the pronunciation guide for Australian and New Zealanders provided by Macquarie University of Australia. The number of incorrect pronunciations were scored and converted to predict Wechsler Adult Intelligence Scale-Revised Verbal IQ scores using the NART manual conversion table (see Appendix G).

6.4 SCREENING INSTRUMENTS AND CLINICAL RATING SCALES

6.4.1 Mini International Neuropsychiatric Interview: Version 5.0.0

The Mini International Neuropsychiatric Interview (MINI) is a brief, structured diagnostic interview of psychiatric disorders in line with the Diagnostic and Statistical Manual of Mental Disorders: Version Four (DSM-IV) (Sheehan et al., 1998). The MINI has become a reference worldwide and has been translated into 65 languages, and demonstrates good concurrent validity. The initial questionnaire contains yes/no questions about psychiatric conditions. If any of these questions are answered yes, a more in-depth interview follows to determine whether a diagnosis can be made (see Appendix I).
The MINI was used in the current study to screen PCOS patients and control participants to ensure that they had not experienced current or past severe psychiatric conditions including bipolar or psychotic disorders. It took approximately 15-20 minutes to administer. Since psychotic disorders were part of exclusion criteria, they were screened over the phone before the participant arrived for baseline assessment.

### 6.4.2 Depression rating scales

Some of the more commonly used depression rating scales in research settings include the HAM-D (M. Hamilton, 1960), MADRS (Montgomery & Asberg, 1979), versions of the BDI (Beck, Steer, & Carbin, 1988), and the Patient Health Questionnaire-9 (PHQ-9) (Kroenke, Spitzer, & Williams, 2001). Since the current study involved a medical sample with endocrinological abnormalities, the HADS was used (see Section 6.4.2.2 for a description). It has been suggested that self-report and clinician-rated scales uniquely contribute to the prediction of treatment outcome (Uher et al., 2012), and thus, both clinician-rated (QIDS-Clinician Rated Scale) and self-report (HADS) scales for depression were included in the current study (see Appendix J).

#### 6.4.2.1 Quick Inventory of Depressive Symptomatology - Clinician Rated

The Quick Inventory of Depressive Symptomatology - Clinician-Rated (QIDS-C) was used in the present study to assess depression symptom severity in both groups. The QIDS is a relatively new and a more time-efficient measure of depressive symptom severity derived from the 30-item Inventory of Depressive Symptomatology (IDS) (Rush, Carmody, & Reimitz, 2000; Rush et al., 2003). The QIDS is sensitive to small changes in mood symptoms related to medications or medical treatments (Rush et al., 2003), making it useful for research purposes. It was originally developed to improve on the available depression rating scales by 1) providing equal weightings (0-3) for each symptom item; 2) providing clearly stated anchors
(duration/frequency numbers) estimating frequency and severity of each item (e.g. 0, 1, 2, or 3), and 3) including DSM-IV criterion items required to diagnose major depression. The usual time-frame for assessing symptom severity is the seven-day period prior to the interview.

The QIDS has good psychometric properties in clinical populations. It shows strong concurrent validity with established depression rating scales (Rush, Bernstein, et al., 2006; Rush, Carmody, et al., 2006), with robust correlations between QIDS-C and the Inventory of Depressive Symptomatology (IDS-C30) for outpatients with MDD (r = 0.82) and bipolar disorder (r = 0.81). Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR) and QIDS-C have identical items and are highly correlated (Bernstein et al., 2009). High internal consistency (r = 0.85) has been demonstrated for QIDS-C (Trivedi et al., 2004). The QIDS-C has been found to be correlate strongly with the HAM-D (r = .61 to .83). An evaluation of QIDS-C in comparison with the MADRS and the QIDS-SR revealed nearly equal Cronbach α reliability (r = 0.85-0.89) (n = 229) (Doraiswamy et al., 2010).

The QIDS has been used in a variety of research and clinical settings including inpatient and outpatient psychiatric clinics and primary care settings. It has been used in study samples including depressed pre-menopausal and post-menopausal females with and without depression (Kornstein et al., 2013; E. Young et al., 2007), females with PCOS (Klimczak et al., 2015; Pastore et al., 2011), depressed post-menopausal females (Kripke et al., 2006), females with varied hormonal status and mood disorders (S. Weiss et al., 2016), and inpatients (70 to 79% females) with MDD and bipolar disorder (Bernstein et al., 2009). The rationale behind using the QIDS in the current study was its sensitivity to small changes in depressive symptomatology over time (Rush et al., 2003), which was relevant for the current study involving anti-androgen treatment.

The scoring system for the QIDS converts responses to 16 separate items into the nine DSM-IV symptom criterion domains, which are used to characterise a major depressive episode. These nine domains include: 1) depressed mood; 2) concentration; 3) self-criticism; 4) suicidal
ideation; 5) interest in social activities; 6) energy/fatigue; 7) sleep disturbance (initial, middle, and late insomnia or hypersomnia); 8) decreased or increased in appetite/weight; and 9) psychomotor agitation or retardation. The total score ranges from 0 to 27 and is obtained by adding the scores of each of the nine symptom domains of the DSM-IV criteria (Rush et al., 2003). Four items are allocated to sleep disturbance (early, middle and late insomnia and hypersomnia), two items to psychomotor agitation and retardation, respectively, four items to appetite and weight disturbance, and one item for the following six domains: depressed mood, decreased interest, decreased energy, worthlessness and guilt, concentration and decision making and suicidal ideation. Each item is rated from 0 to 3 and for symptom domains with more than one item, the highest score obtained on the relevant item for each domain was considered. For example, if early insomnia is 2, middle insomnia is 1, late insomnia is 3 and hypersomnia is 2, the sleep disturbance domain is scored 3. (See Appendix J). The participant was asked to rate the severity and frequency of specific symptoms present over the last seven days. Higher scores reflected more severe depressive symptoms.

6.4.2.2 Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) was developed as a self-report scale to assess, clearly define, and differentiate between depression and anxiety states (Zigmond & Snaith, 1983). It is considered a reliable instrument with valid measures of depressive symptoms and anxiety (Herrmann, 1997), and is sensitive to changes in response to psychological and pharmacological interventions (Cusin, Yang, Yeung, & Fava, 2010). The HADS has been shown to efficiently assess severity of anxiety and depressive symptomatology in both medical and psychiatric conditions (Bjelland, Dahl, Haug, & Neckelmann, 2002; Herrmann, 1997) and shows sensitivity to small changes in symptoms over time in medical conditions (Hinz, Zweynert, Kittel, Igl, & Schwarz, 2009). Assessment of depression in medical populations is complicated due to an overlap between physical symptoms of depression and
symptoms of medical illness or side effects of medications to treat illnesses (e.g., disturbances in sleep and appetite). The HADS does not include items related to the physical symptoms of depression (Cusin et al., 2010; Zigmond & Snaith, 1983), which is why it was selected for inclusion in the present study.

The HADS shows good Cronbach’s α reliability for anxiety ($r = 0.85$) and depression ($r = 0.83$) subscales (Golden, Conroy, & O’Dwyer, 2007). Test-retest reliability of the total scales and subscales is strong, with $r = 0.89$, 0.86 and 0.91 ($p < 0.001$) for anxiety, depression, and total scale scores, respectively (Spinhoven et al., 1997). Overall, the HADS has been found to have strong concurrent validity as shown in a comprehensive review (Bjelland et al., 2002) with comparable sensitivity and specificity of scales such as the General Health Questionnaire, Symptom Checklist-90 (anxiety scale) and the STAI.

The HADS has been used in several studies examining psychological features in females with PCOS and in healthy females (Barry, Kuczmierczyk, et al., 2011; Bazarganipour et al., 2013; Cinar et al., 2011; G. Conway et al., 2014; Dag et al., 2017; Moran et al., 2012; Moran et al., 2010; Moran, Teede, & Vincent, 2015; Zheleznova et al., 2013). Since the current study involved examining and comparing depression and anxiety in females with PCOS and controls, along with assessing change in symptoms within the PCOS group over the course of treatment, the HADS was selected as the self-report measure of psychological symptoms (see Chapter Five).

The HADS determines clinical categories of disorder including no disorder, mild, moderate and severe anxiety/depression. It consists of 14 items with scores ranging from 0 (no symptoms) to 3 (maximum severity) and takes two to five minutes to administer (Snaith, 2003). Results are assessed on each subscale: depression and anxiety, with total scores for each subscale ranging from 0 to 21 and total scores ranging from 0 to 42 points. For both anxiety and depression scales, scores of less than seven indicate non-cases. A score of 8-10 is considered mild, 11-14 is moderate and a score of 15-21 is severe.
An example of an item from the anxiety scale is ‘I get a sort of frightened feeling as if something awful is about to happen’: 0) Not at all, 1) From time to time, occasionally, 2) A lot of the time, 3) Most of the time/very definitely. An example item from the depression scale is ‘I can laugh and see the funny side of things’: 0) As much as I always could, 1) Not quite so much now, 2) Definitely not so much now, 3) Not at all.

6.5 QUESTIONNAIRES FOR ADDITIONAL INFORMATION FROM PARTICIPANTS

6.5.1 Demographic Questionnaire

Different forms of the Demographic Questionnaire were completed by patients with PCOS and control participants at both time-points of the study (see Appendix H). Baseline items covered demographic characteristics such as age, gender, ethnicity, marital status, relationship history, and education. In addition, factors such as current physical illnesses, prescribed medications, current and history of mental illness, history of head injury, menstrual phase, length of menstrual cycle, hearing or sight difficulties, smoking behaviour, alcohol use, substance use and handedness were noted.

At follow-up, the Demographic Questionnaire was a shorter version of the baseline version, as many of the demographic and clinical details did not need to be obtained more than once. The follow-up version included current physical illnesses, medications, phase of menstrual cycle and smoking behaviour.
6.6. COGNITIVE ASSESSMENT

6.6.1 Controlling for factors that may affect cognitive function

Cognitive function is affected by many factors and it is important that consideration is given to these factors during study design and data analysis.

Some of these factors include age, gender, medications, serious psychiatric or neurological illness, menstrual phase, substance consumption and abuse, and state anxiety. In the current study, these factors were taken into account by 1) only including female participants, 2) only including participants aged between 16 and 40 years to avoid hormonal and cognitive effects of menopause and menarche symptoms and to limit the effects of age-related cognitive decline, 3) excluding females with severe neurological and psychiatric illnesses (e.g., multiple sclerosis and bipolar disorder) due to their effects on cognitive function and also since treatment involved (particularly Valproate) stimulates excess androgen production (Isojarvi et al., 1993; McIntyre et al., 2003; Nelson-DeGrave et al., 2004), 4) noting any alcohol/drugs taken by control participants or patients and excluding them if they reported taking any substances prior to testing, and 5) excluding females with other hormonal complications or females who were pregnant. The effects of state anxiety were measured using a Visual Analogue Scale in order to be able to control for state anxiety if significant group differences are found.

6.6.1.1 State anxiety

State anxiety may influence cognitive functioning. Studies have showed an impairment in verbal and spatial processes following induced anxiety (Eysenck & Calvo, 1992; Eysenck & Derakshan, 1998; Vyta, Cornwell, Arkin, & Grillon, 2012). To control for such an effect, a measure of state anxiety was included at both baseline and follow-up cognitive assessments for all participants. State anxiety was measured using a Visual Analogue Scale (VAS), which was completed at three predetermined time-points over the course of cognitive assessment (see
Appendix L). Participants were instructed to rate their current levels of anxiety by drawing a line perpendicularly along the scale from 0cm to 10cm, with 0cm reflecting lowest anxiety (“not at all anxious”) and 10cm reflecting highest possible anxiety (“worst anxiety ever”) (see Figure 6.1). This scale was scored by measuring the number of centimetres between the left-hand end and the participant’s mark (e.g., a mark 5.6cm along the line would equate to a score of 5.6; see Figure 6.1). On the VAS, the interviewer noted the time when the three ratings of anxiety were made. Since this scale is subjectively marked, it is of most value when looking at change within individuals over a certain time period, which is why it was included in the current study.

1. Please rate the way you feel in terms of the dimension given below

2. Regard the line as representing the full range of the dimension

3. Rate your feelings as they are at the moment

4. Mark clearly and perpendicularly across each line

*Example:*

<table>
<thead>
<tr>
<th>Not at all anxious</th>
<th>Worst anxiety ever</th>
</tr>
</thead>
</table>

*Figure 6.1 Visual Analogue Scale used to measure state anxiety at three time-points during the cognitive testing*

6.6.1.2 *Psychoactive substances*

Psychoactive substances have been shown to affect cognitive function. Nicotine has been related to improved performance on cognitive tests, particularly on tasks involving various aspects of attention, working memory and recognition memory (Kumari & Gray, 2003). In
contrast, alcohol and cannabis have a depressogenic effect on the central nervous system which slows reaction time and affects performance on cognitive tasks (Lyvers & Maltzman, 1991; Weissenborn & Duka, 2003). To account for the effects of nicotine, cannabis and alcohol on cognitive test performance in the present study, participants were instructed not to consume alcohol or cannabis within 12 hours prior to the testing session and were asked to report their smoking status, current alcohol and cannabis use and history as a part of the Demographic Questionnaire (see Appendix H).

6.6.1.3 Visual and hearing acuity

Visual and hearing acuity may influence performance on cognitive tests, as most involve presentation of visual or verbal stimuli. Prior to baseline assessment, participants were asked if they had visual or hearing impairments. Most participants who reported visual impairment had their vision corrected either with glasses or contact lenses and wore these during the testing sessions.

6.6.1.4 Practice effects

The term practice effect refers to enhanced performance on cognitive tasks due to prior experience on the same or a very similar task. Better performance occurs due to previous experience of the task rather than actual improvement of the skills being assessed. Practice effects in the present study were minimised in two ways. First, where possible, alternate forms of most tasks were used for the follow-up session. The same version of the RMET, FER and the TMT were used for both test sessions. Overall performance may have improved as a result of participants being familiar with the task, although this is unlikely to occur for the emotion processing tasks since participants did not receive feedback about their baseline performance accuracy. Second, the matched control group completed both testing sessions in order to control for improvement due to enhanced performance strategies.
6.6.2 Cognitive task selection

Cognitive tasks administered in the current study were selected for inclusion because of their ability to measure cognitive functions shown to be impaired in depression, with the addition of tasks shown to be related to PCOS and high androgen levels (refer to Chapter 2, 3 and 4). Several cross-sectional and interventional studies assessing cognitive function in females with PCOS and females with high levels of androgens, or healthy females have focused on verbal and visuospatial learning and memory, verbal fluency, and psychomotor speed since these domains have been shown to be affected by gender differences and hormonal changes in females (Barry et al., 2013; K. Miller et al., 2005; Schattmann & Sherwin, 2007a, 2007b; Shute et al., 1983; Slabbekoorn et al., 1999; Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995) (Refer to Chapter 2, Section 4.9.1 for a description of gender differences in cognitive function). Testosterone administration in females has been positively associated with better performance on tests measuring visuospatial ability and mental rotation (Aleman et al., 2004; Gouchie & Kimura, 1991; Hausmann et al., 2000; Moffat & Hampson, 1996; Postma et al., 2000; Shute et al., 1983) (see Chapter Five) and negatively associated with verbal memory and fluency (Krug, Mölle, Dodt, Fehm, & Born, 2003; Thilers, MacDonald, & Herlitz, 2006). Additionally, testosterone has been shown to be related to emotion processing (van Honk et al., 2005; van Honk & Schutter, 2007; van Wingen et al., 2009). Experimental studies suggest that testosterone administration decreases gaze avoidance and an anxiety response (see Chapter Five), however, little is known about emotion processing and testosterone levels in PCOS samples. Additionally, research suggests significant emotion-specific deficits in MDD (Bourke et al., 2010) and reviews have suggested that individuals with depression show abnormal facial emotion processing (Bouhuys et al., 1999; Bourke et al., 2010; Roiser & Sahakian, 2013; Stuhrmann et al., 2011). On the basis of such findings, psychomotor speed, verbal fluency, verbal learning and memory, visuospatial learning and memory and tasks assessing emotion processing were selected as the focus of cognitive assessment in the current study. The final five broad domains comprised of:
• verbal learning and memory
• visuospatial learning and memory
• attention / executive functioning
• psychomotor speed
• emotion processing

The rationale for using particular cognitive tasks within these domains is discussed in section 6.7.

6.6.3 Order of administration of cognitive tests

All cognitive tests were administered to both groups in the same order for baseline and follow-up (see Table 6.1). This raises the possibility of a systematic bias from test order. Counterbalancing the order of the tests was an option to reduce this bias, however, this was difficult because of the delayed component required in some of the tasks. In addition, during the timed delay of the Consonant Vowel Consonant (CVC) Task, intervening tasks with minimal verbal content were administered to reduce the risk of interference of nonsense word recall on the CVC Task. In the current study, the TMT and the FER Task were administered as intervening tasks after CVC Trials 1-5 and before the CVC direct recall and recognition task was carried out.

As mentioned previously, for some of the cognitive tasks, alternate forms were available for re-testing. Such tasks are indicated by an asterisk in Table 6.1.

6.6.4 Cognitive testing software

The cognitive testing battery was designed to test a broad range of cognitive domains (see Table 6.1). Pen-and-paper tasks were administered according to standardised instructions (Lezak, 2004) and computerised tests according to their manual protocols (CogState and E-Prime manuals). All computerised tasks were presented on a Hewlett-Packard Compaq nc6220 laptop computer.
Table 6.1

Order of Test Administration in the Cognitive Testing Battery

<table>
<thead>
<tr>
<th>Order</th>
<th>Cognitive Test</th>
<th>Cognitive domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Consonant Vowel Consonant Test (CVC) - Immediate recall trials* (1-5)</td>
<td>Verbal learning and memory</td>
</tr>
<tr>
<td>2</td>
<td>Trail-making Test (TMT)(Part A and B)</td>
<td>Psychomotor speed</td>
</tr>
<tr>
<td>3</td>
<td>Facial Expression Recognition (FER) Task</td>
<td>Emotion processing</td>
</tr>
<tr>
<td>4</td>
<td>CVC delayed recall and recognition trials*</td>
<td>Verbal learning and memory</td>
</tr>
<tr>
<td>5</td>
<td>Timed-Chase Test (TCT)*</td>
<td>Psychomotor speed</td>
</tr>
<tr>
<td>6</td>
<td>Groton Maze Learning Test (GMLT) - immediate recall trials*</td>
<td>Visuospatial learning and memory</td>
</tr>
<tr>
<td>7</td>
<td>Controlled Oral Word Association Task (COWAT)*</td>
<td>Executive function / attention</td>
</tr>
<tr>
<td>8</td>
<td>Groton Maze Learning Test (GMLT) - delayed recall trial*</td>
<td>Visuospatial learning and memory</td>
</tr>
<tr>
<td>9</td>
<td>Digit Span Test*</td>
<td>Executive function / attention</td>
</tr>
<tr>
<td>10</td>
<td>Reading the Mind in the Eyes Test (RMET)</td>
<td>Emotion processing</td>
</tr>
</tbody>
</table>

* Indicates cognitive tests with parallel forms for retesting

6.6.4.1 CogState

CogState® Research Software (CogState, 2006) comprises a customisable range of brief computerised cognitive tasks that have been used in a variety of different clinical populations. CogState software (version 5.0.0) was used to present the GMLT and the TCT (© 1999-2006 CogState Ltd.). A computer mouse was used for responses in these tasks. Data were generated and collected automatically as the task was performed and uploaded immediately afterward from the online tool, DataPoint®. CogState has strong construct validity and correlations with standard cognitive tests (Mielke et al., 2014) and has been shown to control for practice effects by administering the tests at short-intervals (Fredrickson et al., 2010; Y. Lim et al., 2013; Maruff et al., 2009).
6.6.4.2 E-Prime

E-Prime (W. Schneider, Eschman, & Zuccolotto, 2002) is a widely-used software in behavioural research and has customisable computerised experiment requirements. The FER Task and the CVC Verbal Task were programmed using the E-Prime 2.0 professional software package. E-studio was used to run these tasks and generated data was saved automatically on the testing computer.

6.7 DESCRIPTION OF TASKS IN THE COGNITIVE TESTING BATTERY

6.7.1 Verbal learning and memory

6.7.1.1 Consonant Vowel Consonant non-word verbal learning Task

The Rey Auditory-Verbal Learning Task (RAVLT) (Rey, 1964) and the California Verbal Learning Task (CVLT) (Delis, Kramer, Kaplan, & Ober, 2000) are two of the most widely used measures of declarative verbal learning and memory. The RAVLT in conjunction with age has been shown to be useful in predicting psychological outcome after trauma to the brain (Ross, Millis, & Rosenthal, 1997) and is a good discriminator between patients with neurological disorders and healthy control samples (Schoenberg et al., 2006). The CVLT can usefully differentiate between patients with mild cognitive dysfunction and healthy control participants (Rabin et al., 2009).

In the current study, the CVC Task was used to assess cognitive function. The CVC Task (Bourke et al., 2012) is a verbal learning and memory task which is designed to be more sensitive in detecting mood-related changes in cognitive function compared with the RAVLT and the CVLT. It is identical in format, administration and scoring to the recall and recognition trials of the RAVLT. The only difference is that in the CVC Task, nonsense words or ‘non-words’ are presented, instead of words with semantic meaning, which lowers the risk of ceiling
effects (Vierck, Porter, Spittlehouse, & Joyce, 2015) and creates greater sensitivity in revealing subtle verbal learning or declarative memory deficits. The CVC Task provides less opportunity compared with traditional verbal memory tasks to access learning and mnemonic strategies (Bourke et al., 2012), thereby providing a mechanism to assess verbal learning and memory while controlling for effects of semantic meaning.

Depressed participants have been shown to score significantly lower on the CVC Task compared with non-depressed healthy participants (Vierck et al., 2015). In another study of depressed outpatients, effect sizes for immediate recall performance on the CVC Task were larger than for the RAVLT, indicating a greater sensitivity to detect deficits in verbal learning (Bourke et al., 2012). Non-words in the CVC Task were selected from the Australian Research Council (ARC) Non-Word Database (Rastle, Harrington, & Coltheart, 2002) and were all monosyllabic in nature, with no semantic meaning in the English language. Each non-word comprised of three letters (e.g., vev, fol, wat) beginning and ending with consonants and with a vowel as the middle letter. Two different lists were used for baseline and follow-up, with a list of 15 words for each testing session (List A and B). Each list consisted of five vowels and a mix of consonants. The vowels in each list appeared three times per list in the same order on List A and B. Pre-recorded lists were played to participants through the laptop, so that participants could see the non-words appear on the screen and could hear them at the same time, with a gap of two seconds. This ensured consistent administration. The first five trials of the CVC Task took approximately 15 minutes and the recognition part of the task about one minute to complete. The number of non-words recalled on each of the learning and memory trials was recorded manually (see Appendix K for recording sheets).

The recognition task was set up to play all 30 List A and List B non-words, and an additional 15 new non-words, which were presented in random order on the computer screen one after the other for up to five seconds. These could be terminated sooner by the participant’s response.
The “Z” key was labelled with a “Y” indicating a non-word from the original list and the “/” key was labelled with an “N” indicating a new non-word. All outcome measures for both the CVC Task recall and recognition tasks were the same as those for the RAVLT. The scores for the recognition task were recorded by the computer.

6.7.2 Visuospatial learning and memory

6.7.2.1 Groton Maze Learning Test

A measure of visuospatial learning and memory was important to include in the cognitive testing battery due to impairment in this domain in depression (Douglas et al., 2011). Visuospatial learning and memory was assessed using the computerised GMLT (CogState, 2006) (Pietrzak et al., 2008; P. Snyder, Bednar, Cromer, & Maruff, 2005; P. Snyder, Werth, et al., 2005). Originally developed by Barker et al. (1931), and modified based on the Hidden Maze Task developed by Milner et al. (1965), the GMLT provides a valid measure of visuospatial processing speed and some aspects of executive function (Pietrzak, Cohen, & Snyder, 2007). The GMLT has been used in studies examining cognitive function in MDD (Douglas et al., 2011), psychotic disorders (P. Snyder et al., 2008), attention deficit hyperactivity disorder (Mollica, Maruff, & Vance, 2004), and mild cognitive impairment (Darby, Maruff, Collie, & McStephen, 2002). Further, the GMLT has been used in interventional studies involving testosterone administration in healthy post-menopausal females (Davison et al., 2011) and pregnant females with anxiety and depression (Kataja et al., 2017), however, this task has not been previously used in studies including females with PCOS. Since the current study involves an examination of the effect of anti-androgen treatment in patients with PCOS, it was imperative that the visuospatial learning and memory measure be sensitive to subtle changes over the course of three months of anti-androgen treatment.
The GMLT consisted of a 10 by 10 grid of square tiles, presented on a computer screen, in which a 28-step pathway was hidden (see Figure 6.2). The tiles at the start and finish were shown in locations at the top left and bottom right of the screen. Participants were instructed to find their way through the hidden pathway, moving one tile at a time. While moving through the hidden maze, participants were required to follow two rules; first, they could not move diagonally and second, they could only move one square at a time. The ability to use standard rules is imperative for optimal learning of the maze (P. Snyder et al., 2008). The computer indicated visual and auditory feedback after each move. If the move was correct the participant was prompted to “go on” by the tone created by making that move along with a green tick mark that appeared under

### Table 6.2

*List A and List B of Nonsense Syllables Included in the Consonant-Vowel-Consonant Task at Baseline and Follow-up to Assess Verbal Learning and Memory*

<table>
<thead>
<tr>
<th>LIST A</th>
<th>Other Words in Recognition Test</th>
<th>LIST B</th>
<th>Other Words in Recognition Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>• vev</td>
<td>• zos</td>
<td>• mov</td>
<td>• zos</td>
</tr>
<tr>
<td>• fol</td>
<td>• mib</td>
<td>• jiz</td>
<td>• mib</td>
</tr>
<tr>
<td>• wat</td>
<td>• lum</td>
<td>• lut</td>
<td>• lum</td>
</tr>
<tr>
<td>• tib</td>
<td>• vad</td>
<td>• dav</td>
<td>• vad</td>
</tr>
<tr>
<td>• pum</td>
<td>• hez</td>
<td>• ked</td>
<td>• hez</td>
</tr>
<tr>
<td>• vob</td>
<td>• nis</td>
<td>• hif</td>
<td>• nis</td>
</tr>
<tr>
<td>• wis</td>
<td>• fav</td>
<td>• zab</td>
<td>• fav</td>
</tr>
<tr>
<td>• lec</td>
<td>• boz</td>
<td>• yoz</td>
<td>• boz</td>
</tr>
<tr>
<td>• mub</td>
<td>• kef</td>
<td>• sen</td>
<td>• kef</td>
</tr>
<tr>
<td>• yaf</td>
<td>• juc</td>
<td>• zup</td>
<td>• juc</td>
</tr>
<tr>
<td>• zet</td>
<td>• gog</td>
<td>• gol</td>
<td>• gog</td>
</tr>
<tr>
<td>• nop</td>
<td>• vit</td>
<td>• vip</td>
<td>• vit</td>
</tr>
<tr>
<td>• jav</td>
<td>• zus</td>
<td>• jum</td>
<td>• zus</td>
</tr>
<tr>
<td>• guz</td>
<td>• jep</td>
<td>• nem</td>
<td>• jep</td>
</tr>
<tr>
<td>• kib</td>
<td>• pab</td>
<td>• caz</td>
<td>• pab</td>
</tr>
</tbody>
</table>
the tile. If the move was incorrect (displayed by a different tone and a red cross), the participant was required to go back and touch the last correct tile and then choose a different direction to advance forward. The GMLT began with three practice trials in a smaller, 5 by 5 grid of squares. This was followed by the main task (10 by 10 grid) which involved finding the hidden pathway over five successive learning trials. During these learning trials, participants were required to construct and hold a spatial representation of the pathway in their working memory.

On each learning trial, the efficiency (number of correct moves per second) was automatically recorded. These scores were automatically saved on the Cogstate software on the laptop, and then transferred to the online data set on Cogstate. Twenty well-matched alternate forms of this test were available which were chosen by the software for each assessment in pseudo-random order to ensure that no participant completed the same hidden path more than once over the course of the study.

![Groton Maze Learning Test initial screen (GMLT, CogState, 2006)](image)

*Figure 6.2*

Groton Maze Learning Test initial screen (GMLT, CogState, 2006)
6.7.3 Attention and Executive Functioning

6.7.3.1 Trail Making Test

The Trail Making Test (TMT) is a commonly used cognitive test which assesses psychomotor speed, attention, and executive function. Under these broad domains, several cognitive functions such as visual search, sequencing, divided attention, cognitive flexibility and conceptual tracking are assessed (Battery, 1944; Mahurin et al., 2006; Partington & Leiter, 1949; SÁNchez-Cubillo et al., 2009).

The TMT consists of two parts (A and B). Part A of the TMT measures visual scanning, number recognition, numeric sequencing, and motor speed. Part B assesses the same cognitive functions as Part A, as well as additional functions of cognitive flexibility and divided attention. Trail Making Test (TMT Part A and B) have been found to have high construct validity (SÁNchez-Cubillo et al., 2009). High inter-rater and alternate forms of reliability have been shown for this test \( r = 0.78 \) to 0.92 (Bowie & Harvey, 2006; Charter, Adkins, Alekoumbides, & Seacat, 1987; Franzen, 1996). The TMT is often used to examine cognitive decline, brain damage (Boll & Reitan, 1973; Giovagnoli et al., 1996; Reitan, 1992) and neurological diseases (Lezak, Howieson, & Loring, 1995; Reitan & Wolfson, 1993; Spreen & Strauss, 1998).

The TMT has been previously used to examine cognitive function in patients with Alzheimer’s Disease (Reitan & Wolfson, 2004), schizophrenia and depression (Mahurin et al., 2006), and obsessive-compulsive disorder (Moritz et al., 2002). State anxiety and gender do not appear to affect performance on the TMT (Chavez, Trautt, Brandon, & Steyaert, 1983). A few studies examining cognitive function in post-menopausal females have included the TMT (Barrett-Connor & Goodman-Gruen, 1999; Kugaya et al., 2003), however, no known studies involving pre-menopausal females with any endocrinological conditions have used this task prior to the current study.
In the current study, Part A and B of the TMT were administered to all participants during both testing sessions. Part A required participants to connect a series of encircled numbers, which were unevenly distributed on the paper, in ascending order. Participants were instructed to join the circles starting from 1-2-3 (to number 25) without lifting their pen/pencil up ‘as quickly as they could’. TMT Part B required participants to connect a series of encircled numbers and letters in an ascending manner, alternating between the two sequences (1-A, 2-B, 3-C to 13-L) (see Appendix M). Most studies use completion time as the only outcome measure for Part A and B because the assessor monitors the performance of the participant and intervenes to correct mistakes at the time of performance, therefore affecting total time taken to complete the task (Hays, 1995; Mahurin et al., 2006; Reitan, 1992; Reynolds, 2002).

Time taken to complete Part A and B of the TMT were recorded and used as outcome variables for this task.

6.7.3.2 Controlled Oral Word Association Test

The Controlled Oral Word Association Test (COWAT) is a measure of verbal fluency and assesses the ability to spontaneously generate words beginning with a given letter within a set time limit. Verbal fluency is a cognitive function that facilitates information retrieval from memory, the success of which requires executive control over processes such as selective attention, self-monitoring and mental set shifting (Lezak, 2004). Evidence suggests that verbal fluency is impaired in MDD (Lezak et al., 1995; Okada, Okamoto, Morinobu, Yamawaki, & Yokota, 2003; Takamura et al., 2016). The COWAT has previously been used in some studies including depressed individuals (Oral et al., 2012; Pu et al., 2015; Roca et al., 2015; Smitherman, Huerkamp, Miller, Houle, & O’Jile, 2007), and in samples with mild cognitive impairment (Petersen et al., 1999). It has been used to detect verbal communication deficits following brain injuries, to measure an individual’s ability to verbally express themselves in
daily life, and to monitor developmental delays related to language in children (Loonstra et al., 2001; E. Strauss, Sherman, & Spreen, 2006).

Some research has shown the COWAT to have a greater female-advantage (Bolla, Lindgren, Bonaccorsy, & Bleecker, 1990; Rodriguez-Aranda & Martinussen, 2006), however, other studies have found mixed results (Lezak et al., 1995; Tombaugh, Kozak, & Rees, 1999). High androgen levels have been shown to be negatively related to COWAT performance in two studies involving females with PCOS (Schattmann & Sherwin, 2007a, 2007b), suggesting a detrimental effect of androgens on verbal fluency. The sensitivity of the COWAT in detecting changes in relation to depression and excess androgens formed the basis for its inclusion in the current study.

The original version of the COWAT included the letters F-A-S which were chosen in a way that was statistically random and no parallel version was produced at the time this test was developed. The newer version, developed as part of Benton and Hamsher’s Multilingual Aphasia Examination (Benton, Hamsher, & Sivan, 1989), provided norms for two sets of letters, C-F-L and P-R-W. These letters were selected based on the frequency of English words beginning with these letters. In these sets, the first letter has a relatively high frequency in the English language, the second has a lower frequency, and the third the lowest frequency of the three. In the current study, C-F-L and P-R-W letter sequences were used at baseline and follow-up respectively. The two forms of the COWAT have good test-retest reliability ($r = 0.83$) (Ruff, Light, Parker, & Levin, 1996).

Additional instructions for the COWAT included not using proper nouns (for example, countries and people’s names), numbers, or the same word with a different ending (for example, eats and eating). Most researchers allow 60 seconds for each of the three word-naming trials in the COWAT, however, in the current study, 90 seconds were allowed to assess verbal slowing. We chose to use the 90 second time-limit in order to be able to compare the current findings with findings from previous studies in more severely depressed samples. Each word was recorded on
a scoring sheet, including mistakes and repetitions. The outcome measure was the total number of words generated for the three trials (see Appendix O for the COWAT recording sheets).

6.7.3.3 Digit Span Test

The Digit Span Test in the Wechsler batteries (intelligence and memory scales) is a widely-used measure of verbal working memory (Lezak, 2004; Wechsler, 1958, 1997). More specifically, it assesses attention and short-term retention capacity (short-term memory) (Lezak et al., 1995) and examines the specific process of temporary storage (Digit Span Forward) and manipulation of information (Digit Span Backward). The Digit Span has been shown to have a high test-retest reliability ($r = 0.83$) (Wechsler & De Lemos, 1981).

The Digit Span Test has been used by several studies examining cognitive function in depressed individuals (Klojčnik, Kavcic, & Vukman; Srisurapanont, Suttajit, Eurviriyanukul, & Varnado, 2017; E. Warren & Groome, 1984) and has been found to be a reliable measure of central executive function in clinically depressed samples compared with healthy participants after controlling for medication status (Channon, Baker, & Robertson, 2009). Since sex hormones have been shown to affect working memory (Janowsky, Chavez, & Orwoll, 2000), assessment of immediate working memory was important to include in the current study.

The Digit Span Test involves two parts (Digits Forward and Digit Backward) requiring attention and short term memory, with a working memory component in the latter test (Lezak, 2004). Participants were instructed to remember up to seven pairs of number sequences and repeat them back to the interviewer in exactly the same way. In Part A, the participant listened to a series of digits, one digit per second, and was asked to repeat it in the same way (Forwards). The first pair of number sequences included three digits, the following pair included one more digit, and so on until the last pair included nine digits (e.g.: 123, 1234, 12345). In the second part of the task (Backwards), the participant was asked to repeat the digits they heard in the
reverse order. This task started with number sequences of two numbers, followed by the next sequence with an additional digit, and so on until the last pair of number sequences included eight digits. Participants advanced when they had correctly recalled either of the sequences of numbers in the seven pairs. The sequences increased in length until a pair of sequences was failed or until the task was completed correctly. Scores on Forward Span as well as Backward Span were added to produce a Total Digit Span score, reflecting total number of digit sequences correctly recalled (maximum 14). Higher scores reflected greater accuracy. Span length (greatest number of digits recalled for Forward and Backward, separately) was also an outcome measure (see Appendix N).

6.7.4 Psychomotor Speed

6.7.4.1 Timed Chase Test

The Timed Chase Test (TCT) was included in the current study to assess visuomotor processing speed (CogState, 2006). This test is generally used as a control task for the motor speed aspect of the GMLT. In this test, the participant was instructed to move as quickly and accurately as possible while following a coloured moving tile, one tile at a time, through the same 10 by 10 grid of grey tiles as presented in the GMLT. The first part of the task contained an untimed practice session to familiarise the participant to the rules of the task. After the practice session, the main trial began, and lasted for 30 seconds. Participants were requested to make as many correct moves as possible within the time period. The number of correct moves made per second during the main timed trial was recorded automatically by Cogstate software and used as the main outcome measure for this test.

6.7.5 Facial Emotion Processing Tasks

The FER Task and the RMET were included in the current study as measures of emotion processing. Emotion processing involves the ability to ‘read the mind’ of individuals by
interpreting social cues and understanding mental states (see Chapter 2). Facial emotion processing is central to human social interactions and is influenced by emotional state (Bourke et al., 2010; Hale, 1998).

Emotion processing is of interest to studies examining mood, since facial expression recognition has been found to be sensitive to pharmacological intervention in depressed individuals (Harmer, O’Sullivan, et al., 2009) and in healthy participants (Harmer, Bhagwagar, et al., 2003; Harmer, Rogers, Tunbridge, Cowen, & Goodwin, 2003). Studies, including reviews, investigating emotion processing in depressed individuals have found impaired facial expression recognition in depressed individuals (Douglas et al., 2011), consistent negative interpretation bias, and an attentional bias towards sad facial expressions and away from happy expressions (Beck, 1979; Bourke et al., 2010; Elliott, Zahn, Deakin, & Anderson, 2011; Mandal & Palchoudhury, 1985). Studies and reviews have shown consistent negative biases for ambiguous or neutral facial expressions in individuals with depression so that positive or neutral faces are likely to be evaluated as more sad or less happy compared with healthy control groups (Bouhuys et al., 1999; Bourke et al., 2010; Douglas & Porter, 2010; Gur et al., 1992; Hale, 1998; Leppänen et al., 2004), which fits with the cognitive theories of depression (Beck, 1979; Fu et al., 2008; M. Warren, Pringle, & Harmer, 2015). This finding is consistent with studies including other stimuli such as negative words or ambiguous sentences in individuals with depression (Hindash & Amir, 2012).

One neuroimaging study has examined facial expression recognition in females with PCOS and found significant changes in brain activity including greater limbic activation during the emotion processing task as found by fMRI in females with PCOS compared with healthy controls (see Chapter Five, section 4.9.2) (Marsh et al., 2013). Other studies have examined facial emotion recognition in relation to hormonal changes during the menstrual cycle in reproductive-aged females without PCOS (C. Conway et al., 2007; Derntl, Kryspin-Exner,
Evidence suggests that testosterone administration in healthy females is associated with a reduced fear response (van Honk et al., 2005), reduced gaze aversion from angry faces (Terburg et al., 2012). Studies have also shown reduced gaze avoidance in females with Social Anxiety Disorder (Enter et al., 2015), and worsened performance on tests of “social intelligence” assessing emotion processing (Olsson et al., 2016; van Honk et al., 2011) following testosterone administration.

6.7.5.1 Facial Expression Recognition Task

The FER Task developed by Harmer and colleagues at Oxford University, United Kingdom (Harmer, Bhagwagar, et al., 2003), was selected for inclusion in the current study to measure facial emotion processing (Harmer, Bhagwagar, et al., 2003; Harmer, Rogers, et al., 2003). This task has been widely used in studies involving depressed individuals (Douglas & Porter, 2010; Harmer, Hill, et al., 2003; Harmer, O’Sullivan, et al., 2009) and in studies involving antidepressant administration in healthy controls (Browning, Reid, Cowen, Goodwin, & Harmer, 2007).

Cognitive models of depression suggest that depression is characterised by impaired facial emotion processing, with studies showing depressed participants to be biased in interpreting neutral faces as negative compared with healthy controls (see Chapter 2) (Douglas & Porter, 2010; Gollan, Pane, McCloskey, & Coccaro, 2008; Gur et al., 1992; Leppänen et al., 2004; Stuhrmann et al., 2011). In order to more thoroughly assess the negative interpretation bias in the current medical sample with PCOS (and with possible symptoms of mild-moderate depression) a greater number of neutral faces were included in the FER Task.

The FER Task features six basic facial emotions; anger, happiness, sadness, fear, surprise and disgust, as well as neutral expressions. These emotions are depicted through images of individual characters’ facial expressions taken from the Pictures of Affect Series (Ekman &
Freisen, 1976). Each facial emotion is morphed between the prototype (full emotion) and neutral by taking a variable percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps (Bhagwagar, Cowen, Goodwin, & Harmer, 2004; A. Young et al., 1997). In the current study, forty-nine instead of thirty neutral facial expressions were included, and facial expressions of surprise (19 faces) were excluded as they were deemed to be least relevant to depressive symptomatology. Furthermore, expression intensities from 50% of each emotion (half way between the full emotion and neutral) to 100% of each emotion were included (see Figure 6.3 for examples of faces presented).

![Figure 6.3]

*Six basic facial emotions; anger, disgust, fear, happiness, sadness and a neutral expression (Harmer, Bhagwagar, et al., 2003)*
During the FER task, faces displaying five basic emotions were presented successively, one after another, on a computer screen for 500ms, followed immediately by a blank screen. A total of 144 facial presentations were presented (49 neutral, and 19 each of angry, happy, sad, fearful and disgusted). Participants were instructed to press one of six labelled buttons on the response pad (NEUTRAL, ANGRY, HAPPY, SAD, FEARFUL and DISGUSTED) as quickly and as accurately as possible. The accuracy and reaction time for each facial emotion was generated and recorded using E-Prime software. This task took approximately 10 minutes for participants to complete.

6.7.5.2 Reading the Mind in the Eyes Test

The Reading the Mind in the Eyes Test (RMET) was used as a measure of emotion processing in the current study (see Appendix P). The RMET (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001; Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997) was originally developed to assess emotion processing in individuals with autism spectrum disorders (Baron-Cohen et al., 2001). Adults with autism spectrum disorders have been shown to perform significantly worse on emotion processing tasks compared with sex-matched controls, and the Autism Spectrum Quotient (a widely used scale to assess autistic traits in adults) has been found to be inversely correlated with scores on the RMET, suggesting an impairment of emotion processing in autism (Baron-Cohen et al., 2001; Wing, 1988). The androgen theory of autism (see Chapter Three, section 3.4.3) (Baron-Cohen, 2002; Baron-Cohen et al., 2005; Baron-Cohen et al., 2011; Baron-Cohen et al., 2001; E. Chapman et al., 2006) provides evidence for a relationship between abnormal levels of androgen and impaired social function due to abnormalities in emotion processing and empathy (Hergüner, Harmanç, Hergüner, & Toy, 2012; Ingudomnukul et al., 2007; Knickmeyer et al., 2006; B. Lee et al., 2017; Ruta, Ingudomnukul, Taylor, Chakrabarti, & Baron-Cohen, 2011; Samson, 2009; Tordjman, Ferrari, Sulmont, Duyme, &
Roubertoux, 1997). This suggests that testosterone may play an important role in emotion processing.

The RMET has been found to have acceptable test-retest reliability (Fernández-Abascal, Cabello, Fernández-Berrocal, & Baron-Cohen, 2013) and has been widely used across different cultures. One study in Italy (Vellante et al., 2013) showed a reliability value of 0.83 and an internal consistency (Cronbach’ alpha) to be 0.60, another study showed a reliability value of 0.81 in a Turkish population for the RMET (Yildrim, Kasar, & Guduk, 2011).

The RMET has previously been used in psychiatric samples including patients with anorexia (Harrison, Tchanturia, & Treasure, 2010), schizophrenia (Kettle, O'Brien-Simpson, & Allen, 2008), borderline personality disorder (Schilling et al., 2012), and in healthy samples (Sapienza, Zingales, & Maestripieri, 2009; Voracek & Dressler, 2006). Furthermore, this test has been used in interventional studies involving testosterone administration in healthy females (Olsson et al., 2016; van Honk et al., 2011).

The RMET includes 36 still pictures of the eye regions of Caucasian individuals, and one practice picture (Autism Research Center, University of Cambridge, Cambridge, United Kingdom) (Baron-Cohen et al., 2001; Baron-Cohen et al., 1997). The main task requires participants to choose one of four adjectives (e.g., “jealous”, “panicked”, “arrogant”, “hateful”) which best described what the person in the picture may have thought or felt (see Figure 6.4 for an example picture) (see Figure 6.4). There was no time limit to complete the task, however, participants were asked to answer as quickly and accurately as they could.

Scoring of the RMET involves a total score resulting from the sum of answers on all 36 items completed by the participant. Previous studies have reported no practice effect (Hallerbäck, Lugnegård, Hjärthag, & Gillberg, 2009; Handford, Lemon, Grimm, & Vollmer-Conna, 2013), therefore, the same task was used at both baseline and follow-up sessions.
6.8 ENDOCRINOLOGICAL/PHYSICAL ASSESSMENT

6.8.1 Physical measures

6.8.1.1 Polycystic Ovarian Syndrome Questionnaire

The Polycystic Ovarian Syndrome Questionnaire (PCOS-Q) was developed for this study by the PhD student and study investigators as a screening tool for volunteers who were responding to publicly posted flyers and newspaper advertisements. This non-standardised measure was used to assess common symptoms related to PCOS. Nine items were included which covered domains related to cosmetic, metabolic, and hormonal irregularities. They were as follows:

- acne,
- excess hair growth on face, back, chest or abdominal region,
- irregular periods/menstrual disturbances,
- weight management problems or weight gain issues,
- male pattern baldness/acute hair loss,
- presence or absence of ovarian cysts,
- history of miscarriages or problems with infertility,
- skin thickening or discoloration (as signs of insulin resistance), and
- family history of PCOS.

The participant’s responses to these questions were the basis of screening for inclusion. If more than three symptoms were marked as ‘yes’ then further participation involved meeting the endocrinologist for accurate diagnosis and appropriate treatment. However, since hirsutism (excess hair growth) is an important and more specific marker of androgen excess, this symptom was given a greater weighting than the other less specific markers of androgen excess, such as weight and acne issues. Additionally, studies have shown that a proportion of females with hirsutism may experience psychological difficulties (Barth et al., 1993; Karjula et al., 2017; Khomami, Tehrani, Hashemi, Farahmand, & Azizi, 2015; Sonino et al., 1993); thus it was considered important to use a specific measure of hirsutism, in order to account for the independent effect that hirsutism may have on mood. The Ferriman-Gallwey score (FG score) was used to evaluate and quantify hirsutism in females with PCOS. This score involved rating the presence of excessive hair from 0 to a maximum of 36 in body areas such as the upper lip, chin, neck, side burns, periareolar area, sternum, and the midline of the lower abdomen. Such criteria used by experts in the field is strict and hence reproducible. Thus, if a participant listed hirsutism without mentioning other issues, they were still considered eligible to meet with the endocrinologist (see Appendix D for the PCOS-Q).

Additionally, for the patients recruited directly through the gynaecological endocrine clinics at Christchurch Women’s Hospital (public hospital) and the Women’s Health Clinic at Southern Cross Hospital (private hospital) in Christchurch, androgen-excess-related symptoms were assessed by the endocrinologist, including:
• visual quantification of hirsutism (FG score)
• acne (mild, moderate, severe)
• BMI
• waist circumference
• body fat percentage
• alopecia (mild, moderate, severe)
• acanthosis nigricans (mild, moderate, severe)
• skin tags (mild, moderate, severe)
• skin thickening (mild, moderate, severe)
• sleep apnea (mild, moderate, severe)
• relevant hormonal levels obtained from routine clinical tests (see Section 6.8.5)

The primary hallmarks of PCOS are hyperandrogenism and anovulation, not ovarian cysts (Dunaif & Thomas, 2001; Sheehan, 2004); thus, ultrasound records of ovarian cysts were obtained only for some patients (at baseline) when deemed necessary.

6.8.2 Androgen measures

Androgen and other hormone levels, as well as symptoms of hyperandrogenism, were collected as part of usual clinical care and was provided by the endocrinologist at baseline. Blood samples were collected from patients to assess androgen measures including Total Testosterone (nmol), Free Testosterone (pmol), and FAI levels. Reference ranges for different types of assays were as follows: 1) Free Testosterone (0.6-6.8 pg/ml [<50 pmol/L]), 2) Total Testosterone (0.1-1.2 ng/ml or 0.3-2.7 nmol/L). In some cases, when Free Testosterone measures were not collected for some patients, FAI levels were used for diagnosis. Blood samples were also collected from patients to assess androgen measures such as DHEA, DHEAS, and other hormonal measures including SHBG, LH, FSH, Estradiol, Progesterone, and AMH were recorded for some patients as deemed appropriate by the endocrinologist.
Blood samples were collected from the control participants by the research nurse at the Department of Psychological Medicine, University of Otago, Christchurch, and were immediately centrifuged and frozen. Samples were then assayed at the Christchurch Health Laboratories and reports were posted to the Department of Psychological Medicine (see Appendix Q). These indicated androgen levels and other hormonal levels including SHBG, LH and FSH, however, other hormonal levels were not the primary hormonal measures of interest.

6.8.3 Treatment

The clinical manifestation of PCOS varies from mild presentation of symptoms such as menstrual irregularities and cosmetic issues to more severe conditions involving reproductive and metabolic abnormalities. In the current study, a treatment plan was put in place by the endocrinologist, based on the individual patient’s symptom profile. Symptoms included:

1) androgen-related symptoms - hirsutism, acne, alopecia, high testosterone levels
2) metabolic symptoms - weight gain issues and insulin resistance
3) reproductive symptoms - irregularities in menstrual cycle

Standard clinical treatment aimed to either decrease and/or block excess androgen levels and involved anti-androgen medication (Badawy & Elnashar, 2011; Falsetti et al., 2000) (see Chapter 4). This included pharmacological agents such as the OCP, CPA, Flutamide, and Spironolactone.

The OCP is effective in regulating the menstrual cycle and in controlling acne by decreasing androgen secretion, decreasing availability of DHT, and increasing SHBG levels (Badawy & Elnashar, 2011; Fenton, 2005; Nader & Diamanti-Kandarakis, 2006). Symptoms of hyperandrogenism varied in nature and severity in the current sample of patients, and were treated by CPA, Spironolactone and Flutamide. The OCP and Spironolactone were used in conjunction in some cases. Daily dosage ranged from 100-200mg for Spironolactone, 25-100mg for CPA (in conjunction with oestradiol) and 250mg for Flutamide. Additionally,
Metformin was used to treat metabolic abnormalities involved in the syndrome, in the form of insulin resistance, and was often used in conjunction with anti-androgen medication (Badawy & Elnashar, 2011; Sam & Dunaif, 2003). In the current study, varied dose regimens of Metformin were used depending on the treatment goal, ranging from 1500-2000mg. In some cases, medication was modified in terms of drug or dosage during the initial 12 week treatment phase, and was noted during the follow-up session.

6.9 PROCEDURE

An overview of the study timeline is presented in Figure 6.5. All participants completed cognitive assessment at the Department of Psychological Medicine, University of Otago, Christchurch, New Zealand. The baseline assessment was conducted between 9am and 5pm on weekdays, and some weekends. For all participants, the baseline assessment began with the demographic questionnaire, followed by the PCOS-Q. Participants in both groups were then administered the MINI, the HADS and the QIDS. The cognitive assessment commenced following the administration of the mood rating scales, and took approximately 90 minutes to complete (see Appendix R for the order of cognitive tests).

Approximately ten weeks after the baseline assessment, participants were contacted by telephone or were e-mailed to arrange a time for the 12-week follow-up assessment. Those patients who were unable to be contacted by telephone or email to organise the twelve-week assessment were posted a letter by six weeks to remind them of the follow-up assessment. At the follow-up assessment, the same procedure was repeated for the cognitive assessment and depression-rating scales, with the exception of the PCOS-Q, the MINI and the NART. No endocrinological assessment was required at this time-point. Therefore, each participant in the PCOS and non-PCOS control groups completed two testing sessions, one at baseline and one approximately 12 weeks after baseline, which added up to 214 assessments in all. On average,
each participant’s follow-up occurred after a mean time of 13.4 (±2) weeks for the PCOS group, and 14.7 (±3) weeks for the control group after their baseline assessment. For both the PCOS and non-PCOS groups, baseline and follow-up interviews were conducted over a period of approximately four years. Participants were financially compensated for travel expenses with $40 petrol vouchers for baseline and follow-up sessions (see Appendix F).

6.10 DATA MANAGEMENT

6.10.1 Assessment of distribution of variables

All continuous variables were plotted using normal residual plots to ensure that they were normally distributed, as assumed in the application of parametric analysis. Variables were found to be normally distributed.

6.10.2 Power calculation and sample size

In the current study, 50 patients with PCOS and 53 control participants provided adequate power (80%) to detect effect sizes of approximately 0.6 (or above) as statistically significant (two-tailed α=0.05).

6.10.3 Computer software

All statistical analyses were conducted using Statistical Packages for Social Sciences (SPSS v25).
<table>
<thead>
<tr>
<th><strong>BASELINE</strong></th>
<th><strong>FOLLOW-UP (12 weeks)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Questionnaires/Interviews</strong></td>
<td><strong>Questionnaires/Interviews</strong></td>
</tr>
<tr>
<td>Demographic Questionnaire</td>
<td>Demographic Questionnaire*</td>
</tr>
<tr>
<td>PCOS-Q</td>
<td>QIDS (Clinician-rated)</td>
</tr>
<tr>
<td>MINI (Screening)</td>
<td>HADS (Self-report)</td>
</tr>
<tr>
<td>QIDS (Clinician-rated)</td>
<td>VAS (Time 0)</td>
</tr>
<tr>
<td>HADS (Self-report)</td>
<td></td>
</tr>
<tr>
<td>VAS (Time 0)</td>
<td></td>
</tr>
<tr>
<td><strong>Cognitive assessment</strong></td>
<td><strong>Cognitive assessment</strong></td>
</tr>
<tr>
<td>NART</td>
<td>CVC-Immediate Recall*</td>
</tr>
<tr>
<td>CVC-Immediate Recall</td>
<td>Trail Making A and B</td>
</tr>
<tr>
<td>Trail Making A and B</td>
<td>Facial Expression Recognition Task</td>
</tr>
<tr>
<td>Facial Expression Recognition Task</td>
<td>CVC-Delayed recall and recognition*</td>
</tr>
<tr>
<td>CVC-Delayed recall and recognition</td>
<td>VAS (Time 1)</td>
</tr>
<tr>
<td>VAS (Time 1)</td>
<td>Timed-Chase Test*</td>
</tr>
<tr>
<td>Timed-Chase Test</td>
<td>GMLT-Immediate trial*</td>
</tr>
<tr>
<td>GMLT-Immediate trial</td>
<td>COWAT*</td>
</tr>
<tr>
<td>COWAT</td>
<td>GMLT-Delayed trial*</td>
</tr>
<tr>
<td>GMLT-delayed trial</td>
<td>Digit Span*</td>
</tr>
<tr>
<td>Digit Span</td>
<td>Reading the Mind in the Eyes Test</td>
</tr>
<tr>
<td>Reading the Mind in the Eyes Test</td>
<td>VAS (Time 2)</td>
</tr>
<tr>
<td>VAS (Time 2)</td>
<td></td>
</tr>
<tr>
<td><strong>Endocrinological assessment:</strong> Androgen and other hormonal measures, and symptoms of hyperandrogenism</td>
<td><strong>Endocrinological assessment:</strong> Androgen and other hormonal measures and symptoms of hyperandrogenism</td>
</tr>
</tbody>
</table>

**Figure 6.5**

Timeline of Study Procedure

PCOS-Q = Polycystic Ovary Syndrome Questionnaire, MINI Screening= Mini International Neuropsychiatric Interview, QIDS = Quick Inventory of Depressive symptoms, HADS = Hospital Anxiety and Depression scale, NART = National Adult Reading Test, CVC = Consonant Vowel Consonant Task, GMLT = Groton Maze Learning Test, COWAT = Controlled Oral Word Association Test.
6.11 STATISTICAL ANALYSIS

General statistical methods are described below. Details relevant to particular outcome measures are outlined in the appropriate sections of Chapters 7 and 8.
6.11.1 Approach to analyses

6.11.1.1 Multiple comparisons

Multiple statistical comparisons were conducted due to multiple outcome measures, thus increasing the risk of Type 1 error. The use of a correction, such as Bonferroni’s correction was considered. However, it was decided to address the risk of Type I errors by: 1) analysing data according to a priori hypotheses related to the cognitive domain that each task tested, 2) including both significant and non-significant results in text, tables and figures to ensure clarity in the number of comparisons, 3) acknowledge that significant results would need confirmation with further research, and 4) using patterns of evidence to facilitate better interpretation of results rather than isolated significant p-values. An alpha significance level set at the conventional two-tailed level of <0.05 was used throughout to indicate statistical significance.

6.11.2 Cognitive data

6.11.2.1 Baseline analysis

Primary analyses

Specific correlations between NART and the following cognitive variables (all p <0.01) were found: between the CVC Trial 5, CVC Total Learning, CVC Delayed Recall, GMLT total errors on Trial 5, GMLT total errors on Trials 1-5, time taken on TMT (Part A and B), COWAT total score, Digit Span Total Forwards and Total Backward Span, and RMET. Body Mass Index (BMI) was also found to be significantly correlated with most cognitive variables, however, since high BMI is a consequence of PCOS, this factor was not controlled for in final analyses.

At baseline, partial correlation coefficients, controlling for the effect of estimated premorbid verbal IQ (assessed by NART) were calculated to determine whether androgen variables were associated with mood, anxiety and cognitive variables across the whole sample. NART was controlled for in the primary analyses and the results with NART are included in Chapter Seven,
while the values in tables and figures without controlling for the effect of NART (raw scores) are presented in the Appendices (see Appendix S). Partial correlation coefficients investigating the associations between androgen variables and emotion processing variables were conducted controlling for mood (HADS-D), as HADS-D was found to be significantly correlated with emotion processing variables. Additionally, analysis also included an examination of the association between mood and cognitive variables, and for this purpose, bivariate correlational analysis was conducted.

**Secondary analyses**

Baseline differences on mood, anxiety and cognitive and emotion processing measures between the PCOS and control groups were analysed using univariate ANCOVA. The effect of estimated premorbid verbal IQ (assessed by NART) was controlled for in these analyses as this differed significantly between the two groups. In univariate ANCOVA (or ANOVA for emotion processing), group (PCOS or control) was a between-participants factor. Regarding emotion processing variables, univariate ANOVA (without NART as a covariate) was conducted as NART was not found to be correlated with facial emotion processing variables.

Effect sizes were calculated for baseline cognitive and emotion processing comparisons between the PCOS and control groups. Estimates of effect size (ES) were calculated using the formula \((\bar{X}_{PCOS\ group} - \bar{X}_{control\ group})/S_{pooled}\).

Multivariate regression analysis was performed on cognitive and emotion processing data for further analysis. Multivariate regression analysis is a technique useful for exploring the relationship between one continuous dependent variable and a number of independent variables or predictors (usually continuous), allowing a sophisticated investigation of the inter-relationship among a set of variables. The current study involved comparative and correlational analyses, and therefore, there was a sound and theoretical reason for conducting more
sophisticated multivariate regression analysis to predict which factors most affect cognitive and emotion processing data. The main focus was to determine whether the association between androgen variables (Free Testosterone and FAI levels) and cognitive variables was independent of factors including BMI, NART, age and HADS score. Analysis included cognitive variables that were found to be significantly correlated with androgen variables in partial correlational analyses, further discussed in Chapter Seven. Analysis comprised of two sets of results, with the inclusion and exclusion of BMI as an independent variable, to clearly elucidate the effect of other independent variables on cognitive variables.

6.11.2.2 Follow-up analysis

Primary analyses

For follow-up analyses, the primary analysis consisted of bivariate correlations examining the association between change in mood and anxiety variables, and change in cognitive and emotion processing variables in the PCOS group. The control group was excluded from this analysis since they were not exposed to any treatment over the period of the study, and therefore, changes would not directly reflect systematic consequences of the treatment. Change in mood and cognitive variables over anti-androgen treatment was calculated by subtracting baseline scores from follow-up scores for each cognitive and mood variable. This change in score was further used in correlational analyses to examine the association between variables. Pearson’s correlation (two-tailed) was used for correlational analyses.

Secondary analyses

Differences between the two groups in change in symptoms of depression, anxiety, cognitive function and emotion processing were assessed using an independent samples $t$-test. Changes within the PCOS group was analysed using paired $t$-tests.
CHAPTER 7

STUDY 1: ASSOCIATIONS BETWEEN ANDROGEN LEVELS AND MOOD, ANXIETY, COGNITIVE FUNCTION AND EMOTION PROCESSING AT BASELINE

7.1 INTRODUCTION

Chapters Two to Five provided a background for the current study by 1) describing major depression and the core features of cognitive impairment and impairment in emotion processing, 2) providing a description of androgens in the context of PCOS, a condition associated with abnormally elevated androgen levels, and 3) a systematic review of studies investigating associations between androgen levels, symptoms of depression and anxiety, cognitive function and emotion processing in females of reproductive age.

7.1.1 Aims

The aims of the current chapter are:

- To determine whether correlations exist between testosterone levels and symptoms of depression and anxiety, across both PCOS and non-PCOS control groups,
- To determine whether correlations are present between testosterone levels and performance on measures of cognitive functioning and emotion processing across PCOS and non-PCOS control groups,
- To determine whether symptoms of depression and anxiety correlate with aspects of cognitive function and emotion processing, and,
• To describe differences between a group of patients with PCOS and a non-PCOS control group on measures of mood, anxiety, cognitive function and emotion processing.

7.1.2 General hypotheses

Hypotheses for this chapter are as follows:

• Free Testosterone and/or FAI levels in females with PCOS will be positively correlated with symptoms of depression.

• Testosterone levels will be negatively correlated with cognitive function, suggesting worse cognitive performance on domains including visuospatial learning and memory, verbal learning and memory, verbal fluency (executive function) and psychomotor speed.

• The PCOS group will show more symptoms of depression compared with the control group.

• The PCOS group will perform less well on tasks assessing specific aspects of cognitive function and emotion processing, which may be related to greater depressive symptomatology, higher androgen levels, or both of these factors.

7.1.3 Chapter outline

The first part of this chapter will present data on recruitment and demographic variables. Following this, data from measures of androgens, mood ratings, and cognitive assessment will be presented as comparisons between the PCOS and non-PCOS control (which will be referred to as simply the ‘control group’ for the remainder of the thesis) groups. Cognitive tasks will be categorised into four main domains: verbal learning and memory, visuospatial learning and memory, attention and executive functioning, and psychomotor speed. Emotion processing findings and social cognition will be presented separately. Finally, results from correlational analyses between these variables will be reported.
7.2 RECRUITMENT STATISTICS

Patients in the PCOS group consisted of:

a) Females who were referred to gynaecological endocrine clinics at Christchurch Women’s Hospital (public hospital) and the Women’s Health Clinic at Southern Cross Hospital (private hospital) in Christchurch, New Zealand \( (n = 32) \). All patients who received information about the study from the treating Gynaecological-Endocrinologist and were considered eligible for inclusion consented to participate in the current study.

b) Females \( (n = 22) \) who were respondents to advertisements in local newspapers (The Christchurch Star Newspaper [http://www.starmedia.kiwi/] and The Metropol [https://www.metropol.co.nz/]) seeking volunteers with untreated symptoms of PCOS.

Over the three-year recruitment period, data were collected from 107 participants, including 54 females with PCOS and 53 control females. The control group consisted of females who were respondents to flyers advertised online [http://www.subjectswanted.co.nz/] and in public places including the University of Otago (Christchurch campus), gyms and sports centres and shopping malls (see Chapter Six, section 6.3.2). Four females in the PCOS group were excluded from the final analysis, as after a more detailed examination by the endocrinologist, their primary diagnosis was found to be CAH and not PCOS.

7.3 PARTICIPANTS

Fifty patients with PCOS and 53 control females were included in the baseline analyses.

Inclusion criteria for the PCOS group was females between the ages of 16 to 40 years, with a diagnosis of PCOS by the treating Gynaecological-Endocrinologist. Regarding the control group, inclusion criteria consisted of reproductive-aged females who showed normal androgen
levels along with no physical symptoms of PCOS such as hirsutism, severe acne or ovarian cysts. Exclusion criteria for both groups was: taking exogenous hormones prior to study, neurological conditions or major chronic medical illnesses (e.g., multiple sclerosis or HIV), current serious alcohol or substance misuse, menopause, pregnancy, previous head injury (loss of consciousness for more than one hour), other endocrinological conditions except for PCOS, current treatment for infertility, insufficient visual or auditory functioning for completion of cognitive tests, and non-fluency in English (see Chapter 6).

Independent-samples t-tests were conducted to compare age (years), BMI, total education and estimated verbal IQ (NART) between the PCOS and control groups (see Table 7.1). There was no significant difference in age ($t = 0.78, p = 0.43$), however, significant differences were found between groups on BMI ($t = 3.12, p = 0.002$), total education ($t = -2.38, p = 0.019$) and estimated verbal IQ ($t = -3.44, p = 0.001$). Since higher BMI is a key diagnostic feature for PCOS and not a separate symptom from the syndrome as such, it was not controlled for in the final analyses. Although attempts were made to match for IQ and education as the groups were recruited, we failed to recruit sufficient number of control participants in a lower IQ and education range. Therefore, in the final analysis, there was a significant difference in the NART score, which was also found to be highly correlated with cognitive variables (see Chapter 6, Section 6.11.2.1), and thus, was treated as a covariate by using analysis of covariance (ANCOVA) in the final analyses.

Distribution of handedness between the two groups did not differ ($\chi^2 = 1.33, p = 0.51$). The New Zealand Census ethnicity categories were used as an outline for determining ethnicity in the sample. PCOS and control groups did not differ significantly in their ethnicity distributions ($\chi^2 = 8.66, p = 0.123$). The majority of both groups identified themselves as ‘European’ which included New Zealand European, English, Dutch, Spanish, and German females. All other
females were categorised as either ‘Maori’, ‘Indian’, ‘Chinese’, or Samoan’, and all were fluent in English.

Table 7.1
Means (SD) or Percentages for Demographic Characteristics in Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>PCOS</th>
<th>Control</th>
<th>t / χ2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)^</td>
<td>28.96</td>
<td>27.85</td>
<td>0.78</td>
<td>0.43</td>
</tr>
<tr>
<td>BMI^</td>
<td>27.80</td>
<td>23.89</td>
<td>3.12</td>
<td>0.002</td>
</tr>
<tr>
<td>Total education (years)^</td>
<td>8.14</td>
<td>9.45</td>
<td>-3.28</td>
<td>0.019</td>
</tr>
<tr>
<td>Verbal IQ (NART)^</td>
<td>105.30</td>
<td>111.04</td>
<td>-3.44</td>
<td>0.001</td>
</tr>
<tr>
<td>Handedness (% right-handed)*</td>
<td>96.00</td>
<td>96.22</td>
<td>1.33</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Ethnicity Data

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>PCOS</th>
<th>Control</th>
<th>t / χ2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>European</td>
<td>42 (84)</td>
<td>40 (75.48)</td>
<td>8.66</td>
<td>0.123</td>
</tr>
<tr>
<td>Maori</td>
<td>4 (8)</td>
<td>2 (3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>2 (2)</td>
<td>5 (9.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samoan</td>
<td>0 (0)</td>
<td>2 (3.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>2 (4)</td>
<td>4 (7.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


7.4 CLINICAL CHARACTERISTICS OF THE POLYCYSTIC OVARIAN SYNDROME GROUP

7.4.1 Androgen levels

Comorbid endocrine disorders in the PCOS group included endometriosis (n = 4) and premenstrual dysphoria (n = 1). Androgen levels including Total Testosterone, Free
Testosterone, FAI and LH levels were significantly higher in the PCOS group compared with the control group (see Table 7.2). Specific androgen measures were obtained for patients based on their clinical assessment by the treating Gynaecological-Endocrinologist, thus, not all androgen values were obtained for all patients with PCOS. For example, for the patients for whom Free Testosterone levels were not collected \((n = 44)\), FAI values were derived from the measurement of Total Testosterone (see Chapter Three, Box 3.1).

**Table 7.2**

*Means (SD) of Raw Scores of Androgen Levels and Other Hormonal Levels in the Polycystic Ovarian Syndrome and Control Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th></th>
<th>Control</th>
<th></th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>43</td>
<td>10.27</td>
<td>7.16</td>
<td>48</td>
<td>6.51</td>
<td>4.65</td>
</tr>
<tr>
<td>FSH</td>
<td>46</td>
<td>5.97</td>
<td>6.95</td>
<td>50</td>
<td>4.66</td>
<td>2.96</td>
</tr>
<tr>
<td>Total T</td>
<td>48</td>
<td>2.04</td>
<td>0.63</td>
<td>50</td>
<td>1.57</td>
<td>0.37</td>
</tr>
<tr>
<td>Free T</td>
<td>44</td>
<td>30.40</td>
<td>14.65</td>
<td>42</td>
<td>18.95</td>
<td>8.49</td>
</tr>
<tr>
<td>FAI^</td>
<td>45</td>
<td>54.35</td>
<td>39.47</td>
<td>42</td>
<td>28.97</td>
<td>17.88</td>
</tr>
<tr>
<td>DHEA</td>
<td>31</td>
<td>7.58</td>
<td>3.35</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SHBG</td>
<td>47</td>
<td>60.93</td>
<td>55.91</td>
<td>42</td>
<td>82.54</td>
<td>73.20</td>
</tr>
</tbody>
</table>

The \(t\)-value for DHEA could not be computed as this androgen measure was only obtained for some PCOS patients but not for the control group. Raw values for hormone levels are presented in this table. LH- Luteinising Hormone, FSH- Follicle Stimulating Hormone, Total T- Total Testosterone (ng/ml), Free T- Free Testosterone (pg/ml), FAI- Free Androgen Index, DHEA- Dehydroepiandrosterone, PCOS- polycystic Ovarian Syndrome, SHBG- Sex Hormone Binding Globulin, **- comparison of log transformed values was conducted due to outliers for statistical comparison, ^- Although the PCOS group was found to show high FAI levels, there was considerable variation in and considerable overlap in FAI levels between the PCOS group and the control group. See Appendix V for a boxplot graph.

**7.4.2 Mood and anxiety comparisons**

Table 7.3 presents scores on the depression and anxiety rating scales in the PCOS and control groups. The mean HADS depression subscale (HADS-D) score was significantly greater in the PCOS group (mean score represented ‘moderate depression’) compared with the control group.
Scores on the anxiety subscale of the HADS (HADS-A) were not significantly different between groups ($t = 1.38, p = 0.171$). The mean QIDS score was significantly higher in the PCOS group compared with the control group ($5.88 \text{ vs } 3.92; \ t = 2.51, p = 0.014$). QIDS scores for the PCOS group indicated ‘mild’ severity of depression whereas for the control group, scores indicated no depression (categorised as ‘none’ according to QIDS scoring) (Rush et al., 2003; Trivedi et al., 2004).

### Table 7.3

*Means (SD) and Ranges for Clinical Characteristics in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>$t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS total score</td>
<td>12.76 7.09</td>
<td>9.49 5.95</td>
<td>2.54</td>
<td>0.013</td>
</tr>
<tr>
<td>HADS anxiety score</td>
<td>7.88 4.02</td>
<td>6.83 3.66</td>
<td>1.38</td>
<td>0.171</td>
</tr>
<tr>
<td>HADS depression score</td>
<td>4.88 3.83</td>
<td>2.66 3.10</td>
<td>3.23</td>
<td>0.002</td>
</tr>
<tr>
<td>QIDS total score</td>
<td>5.88 4.48</td>
<td>3.92 3.37</td>
<td>2.51</td>
<td>0.014</td>
</tr>
</tbody>
</table>

HADS = Hospital Anxiety and Depression Rating Scale. QIDS = Quick Inventory of Depression Symptoms.

### 7.4.3 Axis I Diagnoses

The MINI (Sheehan et al., 1998) was used to assess the presence of Axis I mental disorders in the PCOS and control groups (see Table 7.4). Initial screening ensured that females with schizophrenia, bipolar disorder with psychotic features, and/or substance dependence disorders were not included in the sample. Current psychiatric diagnoses endorsed by PCOS patients included MDD ($n = 2$), impulse control disorder ($n = 1$), SAD ($n = 4$), panic disorder ($n = 2$) and post-traumatic stress disorder ($n = 1$). In the control group, MDD ($n = 6$), GAD ($n = 4$), SAD ($n = 1$), and panic disorder ($n = 2$) were endorsed. Only one patient with PCOS and three
non-PCOS control participants reported using substances (alcohol), however, none met the criteria for a substance dependence disorder. Past psychiatric diagnoses endorsed by PCOS patients included panic disorder \((n = 2)\) and MDD \((n = 4)\). In the control group, MDD \((n = 2)\), panic disorder with agoraphobia \((n = 1)\) and SAD \((n = 2)\) were endorsed.

**Table 7.4**

*Presence of Current Axis I Disorders in the Polycystic Ovarian Syndrome* \((n = 50)\) and Control \((n = 53)\) Groups

<table>
<thead>
<tr>
<th>Axis I Disorders</th>
<th>N (%) PCOS</th>
<th>N (%) Control</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mood disorders</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major Depressive Disorder</td>
<td>9 (18)</td>
<td>6 (11.32)</td>
<td>0.92</td>
</tr>
<tr>
<td>Bipolar Disorder I</td>
<td>3 (6)</td>
<td>1 (1.88)</td>
<td>0</td>
</tr>
<tr>
<td><em>Anxiety disorders</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalised Anxiety Disorder</td>
<td>12 (24)</td>
<td>7 (13.20)</td>
<td>1.99</td>
</tr>
<tr>
<td>Social Anxiety Disorder</td>
<td>10 (20)</td>
<td>4 (7.54)</td>
<td>3.39</td>
</tr>
<tr>
<td>Panic Disorder with Agoraphobia</td>
<td>0</td>
<td>3 (5.66)</td>
<td>0</td>
</tr>
<tr>
<td>Panic Disorder without Agoraphobia</td>
<td>2 (4)</td>
<td>2 (3.77)</td>
<td>0</td>
</tr>
<tr>
<td>Agoraphobia</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Post-Traumatic Stress Disorder</td>
<td>3 (6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Substance Use Disorders</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current alcohol use</td>
<td>1 (2)</td>
<td>3 (5.66)</td>
<td>0</td>
</tr>
<tr>
<td><em>Eating disorders</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulimia nervosa</td>
<td>0</td>
<td>1 (1.88)</td>
<td>0</td>
</tr>
<tr>
<td><em>No diagnosis</em></td>
<td>25 (50)</td>
<td>35 (66)</td>
<td>2.72</td>
</tr>
</tbody>
</table>

PCOS- Polycystic Ovarian Syndrome
7.4.4 Psychotropic medication

At baseline, six control participants reported being on antidepressant medication (SSRIs, n = 4; SNRIs, n = 2). Two control participants reported taking Lorazepam (anxiolytic). One control participant was taking Quetiapine (antipsychotic) for improving mood and sleep. Only one female with PCOS was on antidepressant medication (SSRI) at baseline.

7.4.5 Anti-androgen medication

At baseline, 23 patients and nine control participants reported taking the OCP or the COCP (see Table 7.5). After the Gynaecological-Endocrinologist assessed the symptom profile of patients, changes were made in medication type or dosage, and in some cases, included anti-androgen medication. Anti-androgen medication and hormonal agents included CPA, Spironolactone, Metformin, and Thyroxine, which will be discussed in further detail in Chapter Eight. Two control participants were taking Minoxidil (antihypertensive vasodilator used to treat androgenic alopecia) and Spironolactone, respectively, for dermatological/cosmetic purposes.

Table 7.5
Anti-androgen and Other Hormonal Medication used by the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups at the Baseline Assessment

<table>
<thead>
<tr>
<th>Hormonal medication</th>
<th>PCOS (n = 50)</th>
<th>Control (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>CPA</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>1 (2)</td>
<td>1 (1.89)</td>
</tr>
<tr>
<td>Metformin</td>
<td>5 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>OCP or COCP (Yasmin, Ginette, Brevinor)</td>
<td>23 (46)</td>
<td>9 (16.98)</td>
</tr>
<tr>
<td>IUD (Mirena, Jadelle, Implanon)</td>
<td>4 (8)</td>
<td>5 (9.43)</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>2 (4)</td>
<td>1 (1.89)</td>
</tr>
</tbody>
</table>

OCP = oral contraceptive pill, CPA = Cyproterone Acetate, COCP = combined oral contraceptive pill, IUD = Intra uterine device. Note: One patient reported using Duromine in addition to Metformin.
7.4.6 General medication

A minority of PCOS patients and controls were taking additional medications for conditions such as asthma, eczema, pain, insomnia, hay fever and hypertension. These included pain killers (codeine, ibuprofen, paracetamol, tramadol) (PCOS=4, controls=5), salbutamol inhaler (PCOS=2, controls=1), sleeping medication (melatonin and zopiclone) (PCOS=3, control=0), antibiotics (flucloxacillin, doxycycline) (PCOS=1, controls=2), antihistamines (cetirizine) (PCOS=4, controls=3).

7.5 FACTORS THAT MAY INFLUENCE COGNITIVE FUNCTION

7.5.1 State anxiety

There were no significant differences between groups in reported state anxiety, and the slight reduction in state anxiety observed over time was not significant (see Table 7.6).

Table 7.6
State Anxiety in Polycystic Ovarian Syndrome Group (n = 50) Compared with the Control (n = 53) Group at the Baseline Assessment

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>VAS Baseline</td>
<td>2.11</td>
<td>1.87</td>
<td>1.62</td>
<td>1.61</td>
<td>2.03</td>
<td>0.157</td>
</tr>
<tr>
<td>VAS mid-assessment</td>
<td>-0.13</td>
<td>1.64</td>
<td>-1.67</td>
<td>1.23</td>
<td>0.02</td>
<td>0.899</td>
</tr>
<tr>
<td>(change score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS end of assessment</td>
<td>-0.55</td>
<td>1.66</td>
<td>-0.66</td>
<td>1.32</td>
<td>0.13</td>
<td>0.72</td>
</tr>
<tr>
<td>(change score)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: mid- and end-assessment VAS scores reflect change from baseline, with negative scores representing reduced anxiety. VAS = Visual Analogue Scale, PCOS = Polycystic Ovarian Syndrome, F = univariate ANOVA.
7.5.2 Psychoactive substances

**Nicotine:** The proportion of smokers did not differ significantly between groups ($\chi^2 (1) = 0.24$ $p = 0.62$). Within the PCOS group, there were four smokers (8%), and in the control group, two individuals were recorded as smokers (3.8%).

**Alcohol and cannabis consumption:** Of all participants, only one participant reported consuming cannabis immediately prior to assessment, and was thus, excluded from analysis. Other participants did not report consuming either alcohol or cannabis pre-assessment.

7.6 ANALYSIS OF COGNITIVE DATA

PCOS patients ($n = 50$) and controls ($n = 53$) were compared on baseline cognitive tasks using univariate ANCOVA. Group status (PCOS or control) was the between-participants factor. Analyses included verbal IQ (NART score) as a covariate because of the significant difference found between PCOS and control groups (see Table 7.1), and significant correlations found between NART and some cognitive measures, with the exception of FER Task which showed no suggestion of being correlated with NART (see Chapter Six, Section 6.11.2.1). BMI was correlated with some cognitive variables (see Chapter Six), however, since BMI is a core clinical feature of PCOS, it was decided to not include this variable as a covariate in final analyses.

Estimated marginal means (adjusted means and standard deviations) for all cognitive data are presented in the next sections, generated from ANCOVA (with NART as a covariate). In figures, standard deviations are presented with data values.
7.6.1 Distribution of cognitive variables

Mood, cognitive and emotion processing outcome variables were normally distributed across the entire sample, allowing the use of parametric statistical analysis.

7.7 VERBAL LEARNING AND MEMORY FINDINGS

7.7.1 Consonant-Vowel-Consonant Test: immediate and delayed recall

Univariate ANCOVA was conducted comparing the PCOS group and the control group on Trial 1, Trial 5, Total Learning (Trials 1-5), Delayed Recall and recognition parts of the CVC Test (see Figure 7.1).

No significant differences were found between the PCOS and the control groups after covarying for NART (see Table 7.7).

Table 7.7

Adjusted Means (SD) and Effect Sizes on the Consonant-Vowel-Consonant Task in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>F</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>CVC Trial 1</td>
<td>3.70</td>
<td>1.69</td>
<td>3.57</td>
<td>1.67</td>
<td>0.14</td>
</tr>
<tr>
<td>CVC Trial 5</td>
<td>8.26</td>
<td>3.18</td>
<td>8.73</td>
<td>3.20</td>
<td>0.54</td>
</tr>
<tr>
<td>CVC Total Learning</td>
<td>31.51</td>
<td>11.59</td>
<td>32.19</td>
<td>11.57</td>
<td>0.81</td>
</tr>
<tr>
<td>Delayed Recall</td>
<td>6.68</td>
<td>3.39</td>
<td>6.82</td>
<td>3.42</td>
<td>0.04</td>
</tr>
<tr>
<td>Recognition accuracy List A</td>
<td>13.90</td>
<td>1.13</td>
<td>14.34</td>
<td>1.16</td>
<td>3.39</td>
</tr>
</tbody>
</table>

Adjusted means are presented using NART as a covariate with univariate ANCOVA. F = Univariate ANCOVA, d = Cohen’s d effect size, CVC - Consonant-Vowel-Consonant Task, PCOS - Polycystic Ovarian Syndrome
7.8 VISUOSPATIAL LEARNING AND MEMORY FINDINGS

7.8.1 Groton Maze Learning Test (GMLT) – visuospatial learning

Univariate ANCOVA was conducted comparing the PCOS and the control groups on Trials 1-5 and delayed trials of the GMLT, including NART as a covariate (see Table 7.8). No significant group differences were found on any GMLT variables after controlling for NART.

![Figure 7.1.](image_url)

**Mean (+SD) number of words recalled on the five learning trials and delayed recall trial of the Consonant-Vowel-Consonant Task in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups**

7.8.2 Groton Maze Learning Test (GMLT) (delayed trial) – visuospatial memory

The PCOS and control groups did not differ in the number of errors made on the delayed trial of the GMLT (see Table 7.8, $F = 0.67, p = 0.413$) indicating no specific deficit in visuospatial memory.
Table 7.8

*Adjusted Mean Total Errors (SD) and Effect Sizes for the Groton Maze Learning Test in Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>GMLT Trial 1</td>
<td>15.41</td>
<td>2.96</td>
<td>15.73</td>
<td>2.98</td>
<td>0.28</td>
<td>0.597</td>
</tr>
<tr>
<td>GMLT Trial 5</td>
<td>5.69</td>
<td>3.53</td>
<td>4.73</td>
<td>3.56</td>
<td>1.78</td>
<td>0.185</td>
</tr>
<tr>
<td>GMLT Trials 1-5</td>
<td>44.90</td>
<td>11.94</td>
<td>42.70</td>
<td>11.93</td>
<td>0.83</td>
<td>0.363</td>
</tr>
<tr>
<td>GMLT Delayed Trial/Trial 6</td>
<td>5.12</td>
<td>3.39</td>
<td>4.56</td>
<td>3.34</td>
<td>0.68</td>
<td>0.412</td>
</tr>
</tbody>
</table>

Adjusted means after covarying for NART. *F* = Univariate ANCOVA, *d* = Cohen’s *d* effect size, GMLT - Groton Maze Learning Test, PCOS - Polycystic Ovarian Syndrome

![Figure 7.2](image.png)

*Figure 7.2 Mean (± SD) Total number of errors on the Groton Maze Learning Test (GMLT) over the five learning trials (1-5) and the delay trial in Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups*
7.9 PSYCHOMOTOR SPEED FINDINGS

7.9.1 Timed Chase Test

Univariate ANCOVA showed a significant group difference on the number of correct moves on the TCT (see Table 7.9, \( F = 13.42, p < 0.001 \)) with a moderate effect size (0.76), after covarying for NART (see Table 7.9). The PCOS group performed significantly worse than the control group.

7.9.2 Trail Making Test (Part A)

Univariate ANCOVA showed no significant group differences after covarying for NART on the time taken (\( F = 0.361, p = 0.549 \)) on the TMT (Part A).

Table 7.9

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>F</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCT (no. of correct moves)</td>
<td>43.37</td>
<td>49.18</td>
<td>13.42</td>
<td>&lt;0.001</td>
<td>0.76</td>
</tr>
<tr>
<td>TCT total errors</td>
<td>0.62</td>
<td>0.66</td>
<td>0.03</td>
<td>0.866</td>
<td>0.03</td>
</tr>
<tr>
<td>Trail Making Time Part A (seconds)</td>
<td>20.92</td>
<td>21.69</td>
<td>0.361</td>
<td>0.549</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Adjusted means using NART as a covariate in univariate ANCOVA, \( F = \) Univariate ANCOVA, \( d = \) Cohen’s \( d \) effect size, TCT - Timed Chase Test, PCOS - Polycystic Ovarian Syndrome.

7.10 ATTENTION AND EXECUTIVE FUNCTION FINDINGS

7.10.1 Trail Making Test (Part B)

Univariate ANCOVA showed no significant differences between groups on the time taken on the TMT (Part B) after covarying for NART (\( F = 0.01, p = 0.92 \)) (see Table 7.10).
7.10.2 Controlled Oral Word Association Test

Univariate ANCOVA showed no significant group differences after covarying for NART on the total COWAT score ($F = 3.17, p = 0.07$) (see Table 7.10).

7.10.3 Digit Span

No significant group differences were found in Univariate ANCOVA analysis after covarying for NART on Digit Span variables (Total Forward- $F = 0.33, p = 0.56$), (Forward Span- $F = 0.25, p = 0.62$), (Total Backward- $F = 1.02, p = 0.31$), (Backward Span- $F = 0.27, p = 0.60$) (see Table 7.10).

Table 7.10
Means (SD and SEM) and Effect Sizes on Attention and Executive Function Variables in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT List B (seconds)</td>
<td>44.48</td>
<td>17.81</td>
<td>44.09</td>
<td>17.76</td>
<td>0.01</td>
<td>0.915</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COWAT Total Score</td>
<td>49.53</td>
<td>13.99</td>
<td>54.59</td>
<td>13.97</td>
<td>3.17</td>
<td>0.078</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Total Forward</td>
<td>8.79</td>
<td>2.19</td>
<td>9.04</td>
<td>2.18</td>
<td>0.33</td>
<td>0.567</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Forward Span</td>
<td>6.94</td>
<td>1.20</td>
<td>7.06</td>
<td>1.23</td>
<td>0.25</td>
<td>0.621</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Total Backward</td>
<td>6.87</td>
<td>2.12</td>
<td>7.31</td>
<td>2.11</td>
<td>1.02</td>
<td>0.315</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Span Backward Span</td>
<td>5.00</td>
<td>1.20</td>
<td>5.13</td>
<td>1.16</td>
<td>0.27</td>
<td>0.607</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adjusted means after covarying for NART, $F = $ Univariate ANCOVA, $d = $ Cohen’s $d$ effect size, COWAT- Controlled Oral Word Association Test, TMT - Trail Making Test, TCT - Timed Chase Test, PCOS - Polycystic Ovarian Syndrome

7.11 EMOTION PROCESSING FINDINGS

7.11.1 Facial Expression Recognition Task: recognition accuracy

Since NART was not found to be significantly correlated with the FER Task (see Chapter 6, Section 6.11.2.1), it was not included as a covariate in the final analysis. Univariate ANOVA
found the PCOS group to show significantly worse performance (worse accuracy) in recognising facial expressions of fear ($F = 1.93, p = 0.04$) and sadness ($F = 5.97, p = 0.004$) compared with the control group (see Table 7.11).

7.11.2. Facial Expression Recognition Task: Neutral Misinterpretation Bias

The percentage of neutral faces misinterpreted as one of the five emotions was compared between the PCOS ($n = 50$) and control ($n = 53$) groups.

No significant between-group effects relating to neutral misinterpretation bias were found in univariate ANOVA. Effect sizes of all comparisons on each misinterpreted emotion were small (all ES $< 0.35$) (see Table 7.11).

*Figure 7.3*

Mean (±SD) Recognition accuracy for the five facial expressions of emotional and neutral expressions on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome ($n = 50$) and Control ($n = 53$) Groups
Table 7.11

Mean (SD) of Facial Expression Recognition (Accuracy and Neutral Misinterpretation) Scores, and Effect Sizes in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups

<table>
<thead>
<tr>
<th>Recognition Accuracy</th>
<th>PCOS</th>
<th>Control</th>
<th>F</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anger (%)</td>
<td>58.60 (17.52)</td>
<td>60.09 (15.55)</td>
<td>1.70</td>
<td>0.64</td>
<td>0.08</td>
</tr>
<tr>
<td>Disgust (%)</td>
<td>67.10 (21.54)</td>
<td>71.32 (21.08)</td>
<td>0.05</td>
<td>0.31</td>
<td>0.19</td>
</tr>
<tr>
<td>Fear (%)</td>
<td>86.00 (11.99)</td>
<td>90.28 (9.06)</td>
<td>1.93</td>
<td>0.04</td>
<td>0.40</td>
</tr>
<tr>
<td>Happiness (%)</td>
<td>90.30 (8.77)</td>
<td>90.66 (9.75)</td>
<td>2.01</td>
<td>0.84</td>
<td>0.03</td>
</tr>
<tr>
<td>Neutral (%)</td>
<td>77.20 (16.51)</td>
<td>73.32 (12.84)</td>
<td>1.08</td>
<td>0.18</td>
<td>0.26</td>
</tr>
<tr>
<td>Sadness (%)</td>
<td>61.50 (20.28)</td>
<td>71.79 (14.48)</td>
<td>5.97</td>
<td>0.004</td>
<td>0.58</td>
</tr>
<tr>
<td>FER Total Accuracy (%)</td>
<td>74.20 (8.70)</td>
<td>75.66 (7.49)</td>
<td>0.35</td>
<td>0.36</td>
<td>0.17</td>
</tr>
<tr>
<td>FER Total Reaction Time (%)</td>
<td>1729.70 (261.14)</td>
<td>1762.09 (363.96)</td>
<td>2.98</td>
<td>0.607</td>
<td>0.10</td>
</tr>
<tr>
<td>Neutral Misinterpretation Bias</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral faces misinterpreted as angry</td>
<td>61.96 (25.58)</td>
<td>53.42 (25.08)</td>
<td>0.51</td>
<td>0.092</td>
<td>0.33</td>
</tr>
<tr>
<td>Neutral faces misinterpreted as happy</td>
<td>9.00 (13.60)</td>
<td>6.24 (11.35)</td>
<td>2.12</td>
<td>0.265</td>
<td>0.22</td>
</tr>
<tr>
<td>Neutral faces misinterpreted as sad</td>
<td>17.43 (18.76)</td>
<td>23.45 (21.16)</td>
<td>0.39</td>
<td>0.131</td>
<td>0.30</td>
</tr>
<tr>
<td>Neutral faces misinterpreted as fearful</td>
<td>5.26 (9.23)</td>
<td>6.73 (11.87)</td>
<td>0.82</td>
<td>0.486</td>
<td>0.13</td>
</tr>
<tr>
<td>Neutral faces misinterpreted as disgust</td>
<td>6.33 (11.09)</td>
<td>10.14 (13.80)</td>
<td>1.00</td>
<td>0.127</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*F* = Univariate ANCOVA, *d* = Cohen’s *d* effect size, FER - Facial Expression Recognition, PCOS - Polycystic Ovarian Syndrome
Figure 7.4: Mean (±SD) Misinterpretation of neutral faces for the five facial expressions of emotion on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 50) and Control (n = 53) Groups

7.11.3 Reading the Mind in the Eyes Test

After co-varying for NART, group differences remained significant on the RMET, with the PCOS group performing significantly worse compared with the control group ($F = 8.14, p = 0.005$) (raw score means: $26.78±0.47$ vs $28.10 ± 0.46$; ES = 0.41, total possible score = 36) on RMET.

7.12 CORRELATIONS BETWEEN TESTOSTERONE LEVELS, MOOD RATINGS, COGNITIVE VARIABLES AND EMOTION PROCESSING

Partial correlational analysis was used to explore the relationship between testosterone variables (Total, Free and FAI), mood, and cognitive variables, while controlling for NART across the entire sample ($n = 103$) (since NART was found to be significantly different between the two groups and strongly related to mood and cognitive variables). Partial correlation
analysis was also used to explore the relationship between testosterone levels and emotion processing, however, without controlling for NART, since NART was not found to be significantly correlated with the FER Task. The results with raw scores (before controlling for NART) will be included in Appendix S, and only the adjusted results after covarying for NART will be presented in the following sections.

7.12.1 Correlation between testosterone variables and mood

**Free Testosterone** – Before controlling for NART, significant positive correlations were found between Free Testosterone and both depression measures used in the current study: the depression subscale of the HADS ($r_p = 0.33, p = 0.002$), HADS total score ($r_p = 0.23, p = 0.028$) and QIDS total score ($r_p = 0.24, p = 0.028$) (see Appendix S). No significant correlation was found between the anxiety subscale of the HADS and Free Testosterone levels.

After controlling for NART, no significant correlations were found between mood or anxiety subscales and Free Testosterone levels.

**Free Androgen Index** - Before controlling for NART, significant positive correlations were found between FAI and the depression subscale of the HADS ($r_p = 0.37, p < 0.0001$), HADS total score ($r_p = 0.27, p = 0.011$) and QIDS total score ($r = 0.31, p = 0.004$), but not between FAI levels and anxiety (HADS-A).

After controlling for NART, significant positive correlations remained between FAI levels and the depression subscale of the HADS ($r_p = 0.32, p = 0.002$), HADS total score ($r_p = 0.21, p = 0.05$) and QIDS total score ($r_p = 0.26, p = 0.01$), but not anxiety (HADS-A) ($r_p = 0.15, p = 0.604$).

**Total Testosterone** - No significant correlations were found between any depression or anxiety measures and Total Testosterone levels before or after controlling for NART.
To summarise, after controlling for NART, significant positive correlations were found between higher FAI levels and greater depressive symptomatology in the entire sample including females with and without PCOS ($n = 103$).

Table 7.12

**Correlations between Testosterone Variables and Depression and Anxiety Variables across the Entire Sample ($n = 103$) after Controlling for Verbal IQ (NART)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HADS-D</th>
<th>HADS-A</th>
<th>HADS total score</th>
<th>QIDS Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T (ng/ml)</td>
<td>-0.01</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.01</td>
</tr>
<tr>
<td>Free T (pg/ml)</td>
<td>0.282</td>
<td>0.04</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>FAI</td>
<td>0.32**</td>
<td>0.05</td>
<td>0.21*</td>
<td>0.26**</td>
</tr>
</tbody>
</table>

Table includes adjusted means using NART as a covariate.,* = $p < 0.05$, ** = $p < 0.01$, Positive correlational values indicate an association between higher levels of testosterone and higher symptoms of depression (worse mood, as assessed by the HADS and QIDS). HADS = Hospital Anxiety and Depression Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick inventory of Depressive Symptomatology, FAI = Free Androgen Index, Free T = Free Testosterone (pg/ml), Total T = Total Testosterone (ng/ml).

7.12.2 Correlation between testosterone levels and cognitive variables

Partial correlational analysis was used to study the relationship between testosterone levels and cognitive function while controlling for NART.

7.12.2.1 Verbal learning and memory

After controlling for NART, significant negative correlations were found between Free Testosterone and CVC recognition accuracy ($r_p = -0.23, p = 0.032$) and FAI levels and CVC recognition accuracy ($r_p = -0.20, p = 0.058$). All other correlations were non-significant (see Table 7.13).
Table 7.13
**Correlations between Testosterone Variables and the Consonant Vowel Consonant Task (CVC) Variables across the Entire Sample (n = 103) after Controlling for Verbal IQ (NART)**

<table>
<thead>
<tr>
<th></th>
<th>CVC Trial 1</th>
<th>CVC Trial 5</th>
<th>CVC Total Learning</th>
<th>CVC Delayed Recall</th>
<th>CVC Recognition Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T (ng/ml)</strong></td>
<td>0.00</td>
<td>-0.06</td>
<td>-0.02</td>
<td>0.00</td>
<td>-0.19</td>
</tr>
<tr>
<td><strong>Free T (pg/ml)</strong></td>
<td>-0.03</td>
<td>-0.07</td>
<td>-0.03</td>
<td>0.00</td>
<td>-0.23*</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td>-0.06</td>
<td>-0.07</td>
<td>-0.04</td>
<td>0.00</td>
<td>-0.20*</td>
</tr>
</tbody>
</table>

Table includes adjusted means using NART as a covariate. Positive correlational values indicate an association between higher levels of testosterone and better performance on the CVC Task assessing verbal learning and memory. *p < 0.05, **p < 0.01. CVC - Consonant Vowel Consonant, FAI - Free Androgen Index, Free T - Free Testosterone (pg/ml), Total T - Total Testosterone (ng/ml).

7.12.2.2 Visuospatial learning and memory

After covarying for NART, significant negative correlations were found between Free Testosterone levels and performance on the GMLT (total errors on GMLT Trial 5, \( r_p = -0.246, \ p = 0.023 \); total errors on GMLT Trials 1-5, \( r_p = -0.228, \ p = 0.036 \)) suggesting that higher testosterone levels were associated with worse performance (more errors) on the cognitive task. All other correlations were non-significant (see Table 7.14).

7.12.2.3 Psychomotor speed

After controlling for NART, significant negative correlations were found between Free Testosterone (\( r_p = -0.310, \ p = 0.004 \)) and FAI levels (\( r_p = -0.262, \ p = 0.015 \)) and performance on the TCT (number of correct moves). No significant correlations were found between testosterone levels and performance on the TMT-Part A.
### Table 7.14

**Correlations between Testosterone Variables and Groton Maze Learning Test Variables across the Entire Sample (n = 103) after Controlling for Verbal IQ (NART)**

<table>
<thead>
<tr>
<th></th>
<th>GMLT Errors Trial 1</th>
<th>GMLT Errors Trial 5</th>
<th>GMLT Total Errors Trials 1-5</th>
<th>GMLT Errors Delay Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T (ng/ml)</strong></td>
<td>0.00</td>
<td>-0.16</td>
<td>-0.10</td>
<td>-0.01</td>
</tr>
<tr>
<td><strong>Free T (pg/ml)</strong></td>
<td>-0.15</td>
<td><strong>-0.25</strong></td>
<td><strong>-0.23</strong></td>
<td>-0.07</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td>-0.16</td>
<td>-0.16</td>
<td>-0.16</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Note: Table includes adjusted means using NART as a covariate, positive correlational values indicate an association between higher levels of testosterone and better performance on the GMLT. * = p < 0.05, ** = p < 0.01. **GMLT**- Groton Maze Learning Test, **FAI**- Free Androgen Index, **Free T**- Free Testosterone, **Total T**- Total Testosterone.

### Table 7.15

**Correlations between Testosterone Variables and Psychomotor Speed Variables across the Entire Sample (n = 103) after Controlling for Verbal IQ (NART)**

<table>
<thead>
<tr>
<th></th>
<th>TCT (no of correct moves)</th>
<th>TCT (total errors)</th>
<th>TMT Part A (time)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T (ng/ml)</strong></td>
<td>-0.13</td>
<td>-0.09</td>
<td>-0.10</td>
</tr>
<tr>
<td><strong>Free T (pg/ml)</strong></td>
<td><strong>-0.31</strong></td>
<td>-0.01</td>
<td>-0.14</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td><strong>-0.26</strong></td>
<td>-0.01</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Note: Table includes adjusted means using NART as a covariate, positive correlational values indicate an association between higher levels of testosterone and better performance on the TCT and TMT. * = p < 0.05, ** = p < 0.01. **TCT**- Timed Chase Test, **TMT**- Trail Making Test, **FAI**- Free Androgen Index, **Free T**- Free Testosterone (pg/ml), **Total T**- Total Testosterone (ng/ml).

#### 7.12.2.4. Attention and executive function

After covarying for NART, no significant correlations were found between testosterone variables and attention and executive function variables (see Table 7.16).
Table 7.16

Correlations between Testosterone Variables and Attention and Executive Function Variables across the Entire Sample (n = 103) after Controlling for Verbal IQ (NART)

<table>
<thead>
<tr>
<th></th>
<th>TMT Part B (time)</th>
<th>COWAT Total Score</th>
<th>Digit Span Total Forward</th>
<th>Digit Span Forward Span</th>
<th>Digit Span Total Backward</th>
<th>Digit Span Backward Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T (ng/ml)</td>
<td>-0.19</td>
<td>-0.13</td>
<td>0.00</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.03</td>
</tr>
<tr>
<td>Free T (pg/ml)</td>
<td>-0.10</td>
<td>-0.16</td>
<td>-0.14</td>
<td>-0.18</td>
<td>-0.03</td>
<td>-0.01</td>
</tr>
<tr>
<td>FAI</td>
<td>-0.05</td>
<td>-0.11</td>
<td>-0.10</td>
<td>-0.11</td>
<td>-0.03</td>
<td>-0.00</td>
</tr>
</tbody>
</table>

Note: Table includes adjusted means using NART as a covariate, positive correlational values suggest an association between higher levels of testosterone and better performance on attention and executive function variables, * = p < 0.05, ** = p < 0.01, COWAT - Controlled Oral Word Association Test, TMT - Trail Making Test (Part B), FAI - Free Androgen Index, Free T - Free Testosterone (pg/ml), Total T - Total Testosterone (ng/ml).
7.12.2.5 Correlations between testosterone variables and facial expression recognition and emotion processing task variables

The association between testosterone variables and FER Task and RMET variables was analysed using mood (HADS-D) as a covariate. Univariate analyses showed mood to be strongly correlated with FER variables and with testosterone levels, so it was important to control for this potentially confounding factor.

7.12.2.5.1 Recognition accuracy

After controlling for mood (HADS-D), a negative correlation remained significant between FAI levels and recognition accuracy of fearful faces ($r_p = -0.27, p < 0.05$) on the FER Task suggesting that higher FAI levels were associated with worse recognition accuracy of faces depicting fear (see Table 7.17).

7.12.2.5.2 Reaction time

No significant correlations were found between reaction time on the FER Task and testosterone variables, irrespective of whether mood (HADS-D) as added as a covariate (see Table 7.18).

7.12.2.5.3 Neutral misinterpretation bias

No significant correlations between misinterpretation of neutral faces and testosterone variables were observed before or after controlling for mood (HADS-D) (see Table 7.19).

7.12.2.5.4 Reading the Mind in the Eyes Test

The RMET total score was not found to be significantly correlated with any measures of testosterone levels ($r_p$ values ranged from 0.00 to -0.14, all $p > 0.05$).
Table 7.17  
Correlations between Testosterone Variables and Facial Expression Recognition Variables (Recognition Accuracy) using Mood (Hospital Anxiety Depression Rating Scale- Depression Subscale) as a Covariate across the Entire Sample (n = 103)

<table>
<thead>
<tr>
<th></th>
<th>Angry</th>
<th>Happy</th>
<th>Sad</th>
<th>Fearful</th>
<th>Disgust</th>
<th>Neutral</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T</strong></td>
<td>Raw</td>
<td>-0.08</td>
<td>-0.03</td>
<td>-0.10</td>
<td>-0.02</td>
<td>-0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>Adjusted</td>
<td>-0.04</td>
<td>-0.06</td>
<td>-0.12</td>
<td>-0.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Free T</strong></td>
<td>Raw</td>
<td>-0.07</td>
<td>-0.01</td>
<td>-0.17</td>
<td>-0.06</td>
<td>-0.06</td>
<td>-0.23</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td>Adjusted</td>
<td>0.00</td>
<td>-0.07</td>
<td>-0.19</td>
<td>-0.05</td>
<td>-0.05</td>
<td>-0.04</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td>Raw</td>
<td>-0.07</td>
<td>0.00</td>
<td>-0.21</td>
<td>-0.20</td>
<td>-0.04</td>
<td>-0.21</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>-0.04</td>
<td>-0.06</td>
<td>-0.18</td>
<td>-0.27</td>
<td>-0.04</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

Note: Correlations include raw and adjusted scores for mood (HADS-D); positive correlational values suggest an association between higher levels of testosterone and better performance on the FER Task (recognition accuracy). * = p < 0.05, ** = p < 0.01. **FAI**: Free Androgen Index, **Free T**: Free Testosterone (ng/ml), **Total T**: Total Testosterone (pg/ml).
Table 7.18
Correlations between Testosterone Variables and Facial Expression Recognition Variables (Reaction Time) using Mood (Hospital Anxiety and Depression Rating Scale- Depression Subscale) as a Covariate across the Entire Sample (n = 103)

<table>
<thead>
<tr>
<th></th>
<th>Angry</th>
<th>Happy</th>
<th>Sad</th>
<th>Fearful</th>
<th>Disgust</th>
<th>Neutral</th>
<th>Total Mean Reaction Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>0.00</td>
<td>-0.03</td>
<td>0.00</td>
<td>0.05</td>
<td>0.05</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.04</td>
<td>-0.06</td>
<td>0.01</td>
<td>0.06</td>
<td>0.11</td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Free T (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>0.10</td>
<td>-0.06</td>
<td>-0.08</td>
<td>-0.01</td>
<td>-0.08</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.06</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.08</td>
<td>-0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>0.12</td>
<td>-0.06</td>
<td>-0.05</td>
<td>0.00</td>
<td>-0.10</td>
<td>-0.06</td>
<td>-0.04</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.08</td>
<td>-0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>-0.10</td>
<td>-0.03</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

Note: Correlations include adjusted scores for mood (HADS-D); positive correlational values suggest an association between higher testosterone levels and faster performance on the FER Task (reaction time), FAI- Free Androgen Index, Free T- Free Testosterone (pg/ml), Total T-Total Testosterone (ng/ml).
<table>
<thead>
<tr>
<th></th>
<th>Angry</th>
<th>Happy</th>
<th>Sad</th>
<th>Fearful</th>
<th>Disgusted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/ml) Raw</td>
<td>-0.13</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.16</td>
<td>-0.09</td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.13</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.16</td>
<td>-0.09</td>
</tr>
<tr>
<td><strong>Free T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pg/ml) Raw</td>
<td>0.06</td>
<td>-0.14</td>
<td>-0.11</td>
<td>-0.02</td>
<td>-0.18</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.06</td>
<td>-0.14</td>
<td>-0.09</td>
<td>-0.00</td>
<td>-0.17</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw</td>
<td>0.09</td>
<td>-0.08</td>
<td>-0.18</td>
<td>-0.02</td>
<td>0.20</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.11</td>
<td>-0.07</td>
<td>-0.17</td>
<td>-0.05</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Note: Correlations include adjusted scores for mood (HADS-D), positive correlational values suggest an association between higher testosterone levels and worse performance on the FER Task (neutral misinterpretation bias), indicating more bias in misinterpreting neutral faces.

FAI- Free Androgen Index, Free T- Free Testosterone (pg/ml), Total T- Total Testosterone (ng/ml).
7.12.3 Correlations between mood variables and cognitive variables

The association between mood and anxiety variables and cognitive variables at baseline was assessed by conducting correlational analysis. Findings from these analyses are presented in the following sections.

7.12.3.1 Verbal learning and memory

A significant negative correlation was found between performance on CVC Trial 1 and scores on the HADS-D \((r = -0.208, p = 0.03)\), suggesting that higher symptoms of depression were associated with worse performance on the CVC Task. No other correlations were found to be significant (see Table 7.20).

<table>
<thead>
<tr>
<th>Table 7.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlations between Mood and Anxiety Variables and Consonant-Vowel-Consonant Task Variables across the Entire Sample ((n = 103))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CVC 1</th>
<th>CVC 5</th>
<th>CVC Total Learning</th>
<th>CVC Delayed Recall</th>
<th>CVC Recognition accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS</td>
<td>-0.16</td>
<td>-0.13</td>
<td>-0.13</td>
<td>-0.10</td>
<td>-0.09</td>
</tr>
<tr>
<td>HADS-D</td>
<td>-0.20*</td>
<td>-0.12</td>
<td>-0.15</td>
<td>-0.09</td>
<td>-0.16</td>
</tr>
<tr>
<td>HADS-A</td>
<td>-0.08</td>
<td>-0.10</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.00</td>
</tr>
<tr>
<td>QIDS</td>
<td>-0.16</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.07</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

\*Note: Positive correlational values suggest an association between higher symptoms of depression and better performance on the CVC Task. **\( p < 0.01\), *\( p < 0.05\).  
CVC- Consonant Vowel Consonant Task, HADS- Hospital Anxiety and Depression Rating Scale, HADS-A - anxiety subscale of the HADS, HADS-D - depression subscale of the HADS, QIDS- Quick Inventory of Depressive Symptomatology.
7.12.3.2 Visuospatial learning and memory

A significant negative correlation was found between symptoms of anxiety (HADS-A) and GMLT performance (GMLT Trial 5) \((r = 0.189, p = 0.05)\) indicating that higher symptoms of anxiety were associated with worse performance (more errors made) on the GMLT Trial 5. No other correlations were found to be significant (see Table 7.21).

Table 7.21

<table>
<thead>
<tr>
<th></th>
<th>GMLT 1</th>
<th>GMLT 5</th>
<th>GMLT Total Learning</th>
<th>GMLT Delayed Recall</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS</td>
<td>0.15</td>
<td>-0.13</td>
<td>-0.06</td>
<td>-0.02</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.12</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.09</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.14</td>
<td>-0.18*</td>
<td>-0.11</td>
<td>-0.05</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.15</td>
<td>-0.12</td>
<td>-0.06</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Note: Positive correlational values suggest an association between higher levels of depression and anxiety and better GMLT performance, \(* = p < 0.01, * = p < 0.05\).

GMLT- Groton Maze Learning Test, HADS- Hospital Anxiety and Depression Rating Scale, HADS-A - anxiety subscale of the HADS, HADS-D - depression subscale of the HADS, QIDS- Quick Inventory of Depressive Symptomatology.

7.12.3.3 Psychomotor speed

No significant correlations were found when comparing mood and anxiety variables with psychomotor speed variables (TCT and TMT – Part A) (see Table 7.22).

7.12.3.4 Attention and executive function

The relationship between mood and anxiety variables and attention and executive function variables (TMT-Part B, COWAT, and Digit Span Test) was examined using correlational analysis. Significant negative correlations were found between symptoms of depression
(HADS-D) and performance on Digit Span Total Forward \( r = -0.202, p = 0.04 \) and Digit Span Forward Span \( r = -0.263, p = 0.008 \).

Table 7.22

Correlations between Mood and Anxiety Variables and Psychomotor Variables across the Entire Sample \( n = 103 \)

<table>
<thead>
<tr>
<th></th>
<th>TCT (no of Correct Moves)</th>
<th>TMT Part A (time)</th>
<th>TMT Part A (errors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS</td>
<td>-0.12</td>
<td>-0.03</td>
<td>-0.02</td>
</tr>
<tr>
<td>HADS-D</td>
<td>-0.15</td>
<td>-0.02</td>
<td>-0.03</td>
</tr>
<tr>
<td>HADS-A</td>
<td>-0.06</td>
<td>-0.03</td>
<td>-0.01</td>
</tr>
<tr>
<td>QIDS</td>
<td>-0.04</td>
<td>-0.14</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Note: positive correlational values suggest an association between higher symptoms of depression and anxiety and better performance on psychomotor speed variables.

HADS- Hospital Anxiety and Depression Rating Scale, HADS-A (anxiety subscale of the HADS), HADS-D (depression subscale of the HADS), QIDS- Quick Inventory of Depressive Symptomatology, TCT-Timed Chase Test, TMT-Trail Making Task.

7.13 MULTIVARIATE REGRESSION ANALYSIS

Four variables were associated with cognitive measures on univariate analysis - BMI, NART, age and HADS total score. Therefore, a multivariate regression analysis was conducted to determine whether the association between testosterone variables (Free Testosterone and FAI levels) and cognitive variables was independent of these factors. The inclusion of BMI in this analysis was complex since BMI is part of the syndrome (high androgen levels may also imply high BMI levels in PCOS) and not a separate factor in this context. Therefore, analyses were conducted both with and without BMI.
Table 7.23

Correlations between Mood and Anxiety Variables and Attention and Executive Function Variables across the Entire Sample (n = 103)

<table>
<thead>
<tr>
<th></th>
<th>TMT Part B (time)</th>
<th>COWAT Total Score</th>
<th>Digit Span Total Forward</th>
<th>Digit Span Forward Span</th>
<th>Digit Span Total Backward</th>
<th>Digit Span Backward Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS</td>
<td>0.08</td>
<td>-0.16</td>
<td>-0.20*</td>
<td>-0.18</td>
<td>-0.08</td>
<td>-0.11</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.08</td>
<td>-0.15</td>
<td>-0.25</td>
<td>-0.26**</td>
<td>-0.10</td>
<td>-0.12</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.06</td>
<td>-0.14</td>
<td>-0.11</td>
<td>-0.06</td>
<td>-0.04</td>
<td>-0.07</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.14</td>
<td>-0.12</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.01</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Note: Positive correlational values suggest an association between higher levels of depression and better performance on TMT-List B and Digit Span variables. **= p < 0.01, *= p < 0.05.

COWAT- Controlled Oral Word Association Test, HADS- Hospital Anxiety and Depression Rating Scale, HADS-A- (anxiety subscale of the HADS), HADS-D- (depression subscale of the HADS), QIDS- Quick Inventory of Depressive Symptomatology, TCT-Timed Chase Test, TMT-Trail Making Task.
When multivariate regression analysis included Free Testosterone levels, BMI, NART, Age and HADS-D score, no significant associations were found between Free Testosterone levels and CVC Recognition accuracy, GMLT Total Errors on Trial 5, GMLT Total Errors on Trials 1-5, TCT (number of correct moves), COWAT Total Score and Digit Span Variables (Total Forward and Forward Span).

When analysis included Free Testosterone levels, NART, Age and HADS-D score (excluding BMI), multivariate regression analysis showed a significant negative association between Free Testosterone levels and the TCT (number of correct moves) \( (p = 0.03) \). All other correlations were not significant.

Multivariate regression analyses including FAI levels, BMI, NART, Age and HADS-D score as independent variables found no significant associations between FAI levels and CVC Recognition Accuracy, GMLT Total Errors Trials 1-5 and the TCT (number of correct moves). Associations between FAI levels, NART, Age and HADS-D score and cognitive variables (excluding BMI) from the multivariate regression analysis showed no significant associations.

### 7.14 DISCUSSION

The aims of this chapter were:

- To determine whether testosterone levels were associated with mood, anxiety, cognitive function and emotion processing across the whole sample \( (n = 103) \).
- To determine whether PCOS and control groups differed on measures of mood, anxiety, cognitive function and emotion processing.
- To determine whether the difference between groups on tasks assessing specific aspects of cognitive function and emotion processing was related to greater depressive symptomatology, higher androgen levels, or both of these factors.
The remainder of the current chapter will discuss the main findings in relation to these aims.

7.14.1 Main findings

The main results related to correlations between testosterone levels and mood, cognitive function, and emotion processing variables, after controlling for NART, were as follows:

- Higher testosterone levels (Free Testosterone, FAI) were significantly related to poorer performance on the recognition trial on the CVC Task.
- Higher Free Testosterone levels were significantly associated with worse performance on GMLT Trial 5 and GMLT Trials 1-5.
- Higher testosterone levels (Free Testosterone, FAI) were significantly associated with slower performance on the TCT (number of correct moves).
- After controlling for mood (HADS-D), a significant association remained between higher FAI and worse recognition accuracy of fearful faces on the FER Task.
- Higher FAI levels were significantly associated with worse mood, as assessed by the QIDS and HADS.

In summary, higher Free Testosterone and/or FAI levels were associated with worse performance on several cognitive domains including verbal learning and memory (CVC recognition accuracy), visuospatial learning and memory (GMLT errors), psychomotor speed (number of correct moves on the TCT) and emotion processing (recognition accuracy of fearful faces). Higher FAI levels but not Free Testosterone levels were associated with greater symptoms of depression (see Table 7.24 for an overall summary of findings).

Multivariate analysis was conducted to further determine whether the association between Free Testosterone and FAI levels and cognitive variables was independent of other factors including BMI, NART, age and HADS-D score. Free Testosterone levels were found to be significantly associated with the TCT (number of correct moves) \( p = 0.03 \) when BMI was excluded from
analysis. However, other correlations found in multivariate regression analysis were found to be non-significant.

Comparative analyses showed greater symptoms of depression in the PCOS group compared with the control group. No significant differences were found between groups on symptoms of anxiety. Significant differences were found between the PCOS and control groups on some cognitive measures, including worse performance by the PCOS group on psychomotor speed (TCT), worse (lower) recognition accuracy on fearful faces and worse RMET (social intelligence/empathy) total score.

Analysis examining correlations between mood and cognitive variables showed:

- More severe symptoms of depression (HADS-D) were significantly associated with worse performance on CVC Trial 1.
- Higher anxiety levels (HADS-A) were significantly related to worse performance on the GMLT Trial 5.
- More severe symptoms of depression (HADS) were significantly associated with poorer performance on three Digit Span variables (Digit Span Total Forwards, Digit Span Forward Span, Digit Span Total Score).

Although significant negative correlations were found between Free Testosterone levels and mood variables (HADS, QIDS), correlations were found to be non-significant (at the trend level, $p < 0.10$) after controlling for estimated premorbid IQ (NART). However, FAI levels were found to significantly negatively correlate with mood (higher symptoms of depression (HADS, QIDS)) after controlling for NART. Pertaining to the current study, since high BMI levels are a key symptom of PCOS, we decided not to control for this variable in the final analysis. The inclusion of BMI as a covariate would be problematic since high BMI is a diagnostic marker of PCOS (higher androgen levels may also imply high BMI levels in PCOS) and not a separate factor in this context. However, to further clarify the association between
BMI and HADS depression sub-scale, and keeping in mind the differences in BMI levels between groups, a scatterplot diagram was included to illustrate any potential association (see Appendix T where this scatterplot is presented which shows that the relationship between BMI and HADS is broadly consistent across both groups). Overall, there was a significant association between BMI and HADS ($r = 0.23, p < 0.05$) across groups. Regarding hirsutism scores, studies have found associations between hirsutism scores and poor QoL and social anxiety, but not mood (see Section 4.8.1). Since high testosterone levels are reflective of the condition of hirsutism and since the FG score is a relatively insensitive instrument to examine hyperandrogenism compared with direct assays of testosterone levels (see section 6.8.1.1), we chose not to include FG score in the final analysis. Only just over half of the PCOS sample had an FG score recorded ($n = 27; 54\%$), which made it less likely that a significant correlation would be found, and indeed we did not see evidence of this (see Appendix U). It would be beneficial for future studies with larger samples to investigate this relationship by including sub-group analyses examining mild, moderate and high levels of excessive hair and/or BMI in PCOS groups in relation to mood.

The following section will discuss findings related to cognitive variables in association with previous studies.
7.14.1.1 Associations between mood and anxiety and androgen levels

7.14.1.1.1 Associations between androgen levels and mood

Correlational analyses showed significant positive associations between higher Free Testosterone and FAI levels and greater symptoms of depression (HADS-D, HADS Total Score and QIDS), before covarying for NART. After covarying for NART, significant positive correlations between FAI levels and symptoms of depression remained, however, only an indication of a weak association (trend) was found between Free Testosterone levels and symptoms of low mood.

The relationship between low mood and high levels of Free Testosterone in PCOS is a complex issue (Cooney & Dokras, 2017; Kische et al., 2017). To date, studies examining depression in females with and without PCOS have yielded inconsistent results, including no statistically-significant results, and positive, negative and curvilinear associations between androgen levels, particularly Free Testosterone levels, and symptoms of depression (Adali et al., 2008; Barry, Hardiman, Saxby, & Kuczmierczyk, 2011; Hahn et al., 2005; Hollinrake et al., 2007; Rocco et al., 1991; Weiner et al., 2004). There may be many reasons to explain such variability in results. These may include the presence of unwanted physical symptoms in PCOS including excessive facial hair, cystic acne, higher BMI values, centralised obesity and other skin and hair disorders. Such visible symptoms may have a negative psychological impact on the patient which needs to be taken into account while examining mood symptoms in patients with PCOS (Bishop et al., 2009; Himelein & Thatcher, 2006a, 2006b; Karjula et al., 2017; Kitzinger & Willmott, 2002; Sonino et al., 1993). However, there is mixed evidence regarding mood symptoms in PCOS as some studies have found no association between the physical aspects of the syndrome and symptoms of low mood and anxiety (Barry, Kuczmierczyk, et al., 2011; Barth et al., 1993; Cesta et al., 2016; Shulman et al., 1992). Furthermore, some studies suggest that BMI is associated with low mood in PCOS (Milsom et al., 2013; Pastore et al., 2011) and some of these
results could be explained by the presupposition that the association between Free Testosterone levels and mood may be mediated by BMI, which may be a consequence of PCOS, rather than an independent factor that influences the relationship between androgen levels and mood. Overall, data suggests mixed evidence regarding the association between Free Testosterone or FAI levels and mood.

In the review conducted in the current thesis (see Chapter Five), mixed evidence was found related to the association between mood and testosterone levels. Out of five total studies examining this relationship in non-PCOS females (Bazarganipour et al., 2013; Cinar et al., 2011; Jedel et al., 2011; Moran et al., 2012; Weiner et al., 2004), three studies found a positive association between Free Testosterone/FAI levels and higher symptoms of depression (n = 580), two others found a negative (n = 35) and curvilinear (n = 27) association respectively, while the remaining seven studies found no significant relationship between mood and androgen levels (n = 541) (Annagür et al., 2013; Hollinrake et al., 2007; Klimczak et al., 2015; Livadas et al., 2011; Mansson et al., 2008; Pastore et al., 2011; Rasgon et al., 2003). Overall, studies that found a positive association, similar to the current study, included the HADS, which is more suitable for assessing symptoms of depression in a medical sample and may have helped to assess symptoms of depression more accurately in samples with high androgen levels (as noted in Chapter Five). A meta-analysis showed no relationship between depression in PCOS and elevated Free Testosterone or FAI levels characteristic of PCOS (Cooney et al., 2017). However, this analysis had a strict exclusion criteria (see Chapter Five, section 5.3.1.2). In summary, the finding related to a significant positive association between Free Testosterone and FAI levels and symptoms of depression in the current study may be explained by the inclusion of an adequate sample size and the use of HADS to assess symptoms of depression in a medical population such as PCOS.
Symptoms of depression were found to be significantly greater in the PCOS group compared with control participants, which is consistent with previous studies (Cooney et al., 2017; Dokras, 2012; Hollinrake et al., 2007; Weiner et al., 2004). Overall, there is an agreement concerning the higher prevalence of symptoms of depression in females with PCOS, and a recent review found moderate-severe symptoms of depression in females with PCOS compared with healthy females (Cooney et al., 2017). Using the HADS, which is more suitable for a medical sample such as PCOS, together with the QIDS, the current study found mild-to-moderate severity levels in the PCOS group, in agreement with other studies (Barry, Kuczmierczyk, et al., 2011; Veltman-Verhulst et al., 2012), however, these findings are inconsistent with Cooney et al.’s (2017) review. Additionally, Axis I disorders (MINI, see Table 7.4) were found to be more prevalent in females with PCOS, particularly mood disorders (MDD and bipolar disorder), and anxiety disorders (particularly GAD and SAD). Current symptoms of anxiety (HADS-A) in the current study were not found to be significantly different between the two groups, which is in contrast to studies documenting higher rates of symptoms of anxiety in females with PCOS (Cooney & Dokras, 2017; Dokras et al., 2012). The control group in the current study was, however, not randomly selected, and this limitation will be discussed in detail in the final section (see Section 7.15.1.3) of the current chapter.

Inconsistent findings may also be a result of including measures designed to detect different symptom severity levels. For example, in a large study (226 females) (Cinar et al., 2011), higher FAI levels were found to be associated with greater depression severity as measured by the HADS. The HADS was primarily designed to determine the severity of depression, not to determine whether caseness is met. The HADS together with the QIDS was considered suitable for assessing mood and anxiety in the current medical sample (see Chapter 6, Section 6.4.2 for the rationale behind choosing these rating scales). The HADS was designed to assess depressive symptoms in patients with medical illnesses. The QIDS was designed to detect more severe
symptoms of depression, and in the current study was used a) as an interviewer rated scale, which may be less subject to the influence of physical symptoms, and b) in case depressive symptoms were in the more severe range. Previous studies have found no association between mood and androgen levels using the QIDS (Klimczak et al., 2015; Pastore et al., 2011) which could be due to lesser severity of symptoms involved in PCOS compared with psychiatric samples. Some previous negative studies have extrapolated depression severity from scales which are not designed to measure clinical levels of depression. For example, studies have used Quality of Life measures and have found higher levels of depression, anxiety and emotion distress in patients with PCOS compared with controls (Hahn et al., 2005; Veltman-Verhulst et al., 2012).

To summarise, in the current study, symptoms of depression but not anxiety were found to be higher in the PCOS group compared with the control group. Additionally, higher levels of Free Testosterone and FAI levels, characteristic of PCOS, were found to be significantly positively correlated with symptoms of depression across the entire sample. The current study, therefore, provides evidence that higher symptoms of depression are associated with raised testosterone levels in females, independent of the presence of PCOS.

7.14.1.2 Associations between cognitive function and androgen levels: Group comparisons and correlation analyses

7.14.1.2.1 Verbal learning and memory

Free Testosterone and FAI levels were significantly negatively correlated with recognition accuracy on the CVC Task, after controlling for NART, suggesting that higher Free Testosterone and FAI levels were associated with worse performance on the recognition trial of the CVC Task. No significant differences on CVC variables were observed between the two groups before or after co-varying for NART.
Seven cross-sectional studies have examined verbal learning and memory in relation to androgen levels in healthy females, females with PCOS and female-to-male transsexuals (Gómez-Gil et al., 2009; Halari et al., 2005; Hussain et al., 2016; Janowsky et al., 1998; S. Phillips & Sherwin, 1992; Romero-Martínez et al., 2015; Schattmann & Sherwin, 2007b). One study including females with PCOS found a significant difference between PCOS and the healthy control groups on verbal learning and memory measures (Schattmann & Sherwin, 2007b). Schattmann and Sherwin (2007b) reported that their PCOS group ($n = 28$) performed significantly worse than their healthy control group ($n = 20$) on one measure of verbal memory (Paired Associates Test and Logical Memory) but not the other (RAVLT). However, in this study, no significant correlations were found between androgen levels and verbal learning and memory measures. Two further studies have shown significant yet varied results (S. Phillips & Sherwin, 1992; Romero-Martínez et al., 2015). Phillips and Sherwin (1992) in 25 females, found a significant negative correlation between Free Testosterone levels and one sub-test of verbal memory (Delayed Paragraph Recall; but not Logical Memory, Visual Reproduction, and Associate Learning). In another small study (Romero-Martínez et al., 2015), a curvilinear relationship was found between Free Testosterone levels and verbal memory (RAVLT) in caregiving mothers of children with autism. Increases in testosterone levels significantly correlated with better verbal memory, up-to a certain point, followed by reduced performance with increasing testosterone levels only in the group with caregivers ($n = 24$) but not the control group, comprising of 22 caregiving mothers of children without autism.

Other cross-sectional studies examining verbal learning and memory in relation to testosterone levels have found non-significant results (Gómez-Gil et al., 2009; Halari et al., 2005; Hussain et al., 2016; Janowsky et al., 1998). It is to be noted that one study with non-significant findings measured Total Testosterone levels and not Free Testosterone levels (Hussain et al., 2016). Baseline results from one interventional study failed to find a significant correlation between
Free Testosterone and FAI levels and verbal learning and memory (Paired Associates Test, Logical Memory and RAVLT) (Schattmann & Sherwin, 2007a).

In the current study, only one aspect of verbal learning and memory was found to be associated with testosterone levels. Thus, although it is likely that this may be a chance finding, it could be that a specific impairment in recognition memory (and not recall) may be associated with testosterone levels. Little research in has examined the specific association between recognition memory and testosterone levels. Furthermore, the CVC Task is challenging as it provides less opportunity compared with traditional verbal memory tasks to access learning and mnemonic strategies (Bourke et al., 2012) and, therefore, may be more sensitive to show small and significant results.

7.14.1.2.2 Visuospatial learning and memory

The GMLT was used as a measure of visuospatial learning and memory. Correlational analyses showed that Free Testosterone was associated with worse performance (more errors) on some variables from the GMLT (total errors on Trial 5 and Trials 1-5, but not the Delayed Trial), which remained significant after controlling for the NART. No significant group differences were found on any GMLT variables (total errors for Trial 1, Trial 5, Trials 1-5 or Delayed Recall) before or after controlling for NART.

There is very little data available regarding the relationship between visuospatial learning and memory in females with PCOS, particularly in relation to testosterone levels. On the other hand, visuospatial ability has been examined by numerous studies. Mental rotation ability has been found to be positively associated with testosterone levels in interventional studies involving testosterone administration (Aleman et al., 2004; K. Miller et al., 2005; Pintzka et al., 2016; Slabbekoorn et al., 1999; Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995).
(see Chapter Five). However, visuospatial ability is a separate function from spatial learning and memory, and only the latter was assessed in the current study.

Only three studies so far have examined visuospatial learning and memory in relation to testosterone levels in females, with inconsistent results (Gómez-Gil et al., 2009; Janowsky et al., 1998; Schattmann & Sherwin, 2007b). One cross-sectional study, including 33 female-to-male transsexuals (Gómez-Gil et al., 2009), found significantly better scores on visuospatial memory (Visual Paired Associates Test) in the testosterone group \((n = 9)\) compared with the control group \((n = 10)\). Janowsky et al. (1998) in a longitudinal observational study found no significant association between visuospatial memory (Toy Task) and Free Testosterone levels in 30 healthy females. In another cross-sectional study (Schattmann & Sherwin, 2007b) including a sample of 28 females with PCOS and 20 healthy controls, no significant correlations were obtained between testosterone levels and spatial memory (Block-Tapping Task). Post-hoc analyses showed significant group differences on only one component of the Block-Tapping Task (the Backward component of immediate memory, but not the Forward component), with the PCOS group showing significantly worse performance compared with the healthy control group. The Block Tapping Task is best suited to examine short-term visuospatial memory in individuals (Kessels, van Zandvoort, Postma, Kappelle, & de Haan, 2000) and is similar to the early learning trials involved in the GMLT, however, is unlike the remainder of the task, which may help explain the difference in results between Schattmann & Sherwin’s (2007b) study and the current findings including a significant negative association between Free Testosterone levels and worse performance on the GMLT.
7.14.1.2.3 Psychomotor speed

Free Testosterone and FAI levels were found to be significantly negatively correlated with performance on the TCT (number of correct moves) after controlling for NART, suggesting that higher testosterone levels were associated with worse performance (fewer correct moves) made on the TCT. Additionally, multivariate regression analysis found Free Testosterone levels to be significantly associated with the TCT (number of correct moves) ($p = 0.03$) when BMI was excluded from analysis, and this was the only task which was found to be significantly associated with testosterone levels. The PCOS group showed significantly worse performance on the TCT (fewer number of correct moves) after controlling for NART, with a large effect size (0.76). No significant correlations were found between testosterone levels and performance on the TMT– Part A (time). No significant group differences were found between the two groups on the remaining psychomotor speed variables after controlling for NART.

In explaining this finding, it could be that that higher Free Testosterone and FAI levels characteristic of PCOS have a negative effect on psychomotor speed, which is considered a female-superior cognitive domain. Indeed, previous studies have found a significant female-advantage in tasks assessing psychomotor speed (Gouchie & Kimura, 1991; Halpern, 2000) and it may be that abnormally elevated androgen levels negatively affect performance suggesting an organisational effect of testosterone. However, most studies examining psychomotor speed in reproductive-aged females with and without PCOS do not suggest consistent results (Gouchie & Kimura, 1991; Janowsky et al., 1998), except for one robust, cross-sectional study which found significant group differences and a significant negative correlation between FAI levels and psychomotor speed (Schattmann & Sherwin, 2007b). Schattman and Sherwin (2007b) reported that their PCOS group with high FAI levels ($n = 29$) performed significantly more slowly than their healthy control group ($n = 22$) on one measure of motor speed, manual dexterity (Purdue Pegboard), but not the other measure of perceptual speed (Finding A’s). Additionally, FAI levels were found to be negatively correlated with performance on the
manual dexterity task. However, in this study, similar to the current study, multiple cognitive tests were included which is a methodological limitation. Another study (Gouchie & Kimura, 1991) examining psychomotor speed (Finding A’s Test) found no significant differences between two groups including females with high and low Free Testosterone levels and found no significant correlations between androgen levels and cognitive performance in these groups. Janowsky et al. (1998) found a significant positive correlation between Free Testosterone levels and the Dart Throwing Task which assessed visuo-motor speed, however, only in females using their dominant hand, which is likely to be a chance finding.

The results of the current study are similar to those of Schattman and Sherwin’s study (2007b), possibly due to the inclusion of the motor task involved but also due to the inclusion of females with PCOS with high FAI levels in contrast to the other studies including only healthy females. Testosterone levels in these study samples may not be as elevated compared with females with PCOS, which may limit the spread of androgen levels and the chance of finding significant associations.

In the current study, the TCT was used as a measure of motor speed, while the TMT-A was used to assess the additional perceptual processing component. In contrast to the TCT, no significant difference in performance was observed on the TMT-A in the PCOS group compared with the control group. Although these findings may seem contradictory, it may be that these two tests have a subtle difference in their requirement of different strategies and cognitive processes.

In summary, current findings included significant negative correlations between Free Testosterone and FAI levels and TCT performance (number of correct moves) and significantly worse performance by the PCOS group compared with the control group. This suggests that high testosterone levels may be negatively associated with motor speed performance. It may be that testosterone levels are negatively associated with the motor speed aspect of psychomotor
speed rather than the perceptual speed component which previous studies have failed to find (Gouchie & Kimura, 1991; Schattmann & Sherwin, 2007b).

7.14.1.2.4 Attention and executive function

No significant correlations were found between testosterone levels and attention and executive function variables after controlling for NART. The PCOS group did not show significantly different performance to the control group on measures of attention and executive function (TMT - Part B, COWAT, and Digit Span) after controlling for NART.

Relatively little information is available regarding the relationship between attention and executive function and testosterone levels in females. However, available data suggest that higher testosterone levels may be associated with worse verbal fluency (executive function) (Krug et al., 2003; Thilers et al., 2006). Only one cross-sectional study found a significant relationship between executive function and testosterone levels in females. In a cross-sectional study examining verbal fluency in reproductive-aged females with and without PCOS (Schattmann & Sherwin, 2007b), females with PCOS and high Free Testosterone levels \((n = 29)\) demonstrated significantly worse performance on verbal fluency (COWAT) compared with healthy females \((n = 22)\). Furthermore, FAI levels were negatively correlated with COWAT scores suggesting high testosterone levels may dampen executive functioning, which the current study did not find. Other cross-sectional studies have found no significant association between executive function measures and androgen levels in healthy females (Halari et al., 2005; Hassler et al., 1992; Janowsky et al., 1998; McKeever et al., 1987; Moffat & Hampson, 1996; Neave et al., 1999).

To summarise, no significant correlations were found between attention and executive function performance and testosterone levels in the current study. Additionally, no significant group differences were found between groups on these measures.
**7.14.1.3 Associations between emotion processing and androgen levels**

**7.14.1.3.1 Facial Expression Recognition - Recognition accuracy and neutral misinterpretation**

Significant negative correlations were found between Free Testosterone and FAI levels and recognition accuracy of neutral and sad facial expressions, as well as total accuracy on the FER Task. However, after controlling for mood HADS-D, only the negative correlation between FAI levels and recognition accuracy of fearful faces remained significant. That is, higher FAI levels were associated with worse recognition accuracy of fearful faces. Correlational analyses showed no significant relationship between misinterpretation bias of neutral faces and testosterone levels. The PCOS group \((n = 50)\) showed poorer recognition accuracy of fearful faces \((ES = 0.40)\) and sad faces \((ES = 0.58)\) compared with the control group \((n = 53)\).

Sex hormones can influence emotional states and their expression in individuals. Testosterone has been mainly associated with emotions of anger, and related behaviours such as aggression and social dominance (van Wingen, Ossewaarde, Bäckström, Hermans, & Fernández, 2011), however, this has been studied mostly in males (Archer, 2006; Mazur & Booth, 1998). Until now, the focus of most research on emotional processing in relation to sex hormones in females has focused on menstrual fluctuations in oestrogen and progesterone, and the effect of these fluctuations on mood (Poromaa & Gingnell, 2014) and on the perception of sexual or reproductive stimuli involved in emotion processing (Krug, Plihal, Fehm, & Born, 2000; Macrae, Alnwick, Milne, & Schloerscheidt, 2002). The majority of studies examining emotion processing in females with PCOS and in healthy control samples have used an interventional study design involving testosterone administration (Bos et al., 2016; Bos et al., 2013; Enter et al., 2015; Hermans et al., 2007; Olsson et al., 2016; Terburg et al., 2012; van Honk et al., 2005; van Wingen et al., 2011; van Wingen et al., 2009). Findings pertaining to these studies will be discussed in the following chapter (Chapter Eight) focusing on the longitudinal (post-treatment) results of the current study.
Only two cross-sectional studies until now have investigated the relationship between testosterone levels in reproductive-aged females and facial emotion processing performance (Stanton et al., 2009; van Honk et al., 1999). Stanton et al. (2009) used fMRI to measure blood-oxygen-level dependent (BOLD) responses to angry faces (Face Stimulus Test) in the amygdala and ventromedial prefrontal cortex, as a function of endogenous testosterone in 14 healthy females. Free Testosterone levels were not associated with BOLD responses during the emotion processing task in this study. van Honk et al. (1999) in their observational study involving multiple time-coursed samples of salivary Free Testosterone obtained from 16 healthy females found significant associations between Free Testosterone levels taken six-hours prior to testing (higher levels of testosterone compared with the samples at other time points thereafter) and higher selective attention to angry faces (Emotional Stroop Task) and between the same time-lag in testosterone samples and mood (POMS subscales including anger and tension). While these results may be due to chance, findings suggest that high levels of testosterone in females are associated with a reduction in sensitivity and higher selective attention to angry faces. One small neuroimaging study examining emotional processing in twelve females with and without PCOS with insulin resistance found greater limbic activation (including the prefrontal cortex, anterior cingulate, amygdala, and ventral basal ganglia/nucleus accumbens) in the PCOS group \((n = 7)\) during an emotion processing task compared with non-insulin resistant controls \((n = 5)\) (Marsh et al., 2013). However, this study did not report differences between groups in performance on tasks assessing emotion processing.

Literature suggests an association between testosterone and aggression and studies have shown high testosterone levels to be positively associated with aggression (J. Harris et al., 1996). This may be due to a possible activating effect on the limbic region, particularly on the amygdala, observed through increased neural activity (Derntl et al., 2009; van Wingen et al., 2010; van Wingen et al., 2009), or through metabolisation into neuroactive steroids (Aikey, Nyby, Anmuth, & James, 2002) or aromatisation to oestrogen in presynaptic terminals (Hermans,
Ramsey, & van Honk, 2008; van Wingen et al., 2010), which may also have an effect on amygdala or limbic activation, thought to be important in fear perception (Adolphs, 2008). In contrast to findings related to greater amygdala activation associated with testosterone levels, studies have found testosterone to be associated with a reduced central fear and stress responses (Hermans et al., 2006; van Honk et al., 2005) and to selectively reduce sensitivity toward threatening emotional expressions, observed through reduced conscious recognition of facial threat, including emotions of fear, anger and disgust (van Honk & Schutter, 2007; Wirth & Schultheiss, 2007). Furthermore, some animal and human studies have shown that testosterone down-regulates the stress response (Aikey et al., 2002; Bitran, Kellogg, & Hilvers, 1993) with the involvement of the amygdala (De Kloet, Joëls, & Holsboer, 2005). Testosterone has also been linked with worse empathy (Olsson et al., 2016; van Honk et al., 2011), which is a crucial function for understanding or inferring the emotional states of others, important for healthy social functioning. It has been postulated that testosterone, being predominantly a male hormone, may be associated with diminished or impaired empathic responses observed in individuals with autism, mostly occurring in males (Baron-Cohen et al., 2011; Manning et al., 2001) which suggests an organisational effect of this androgen. In the current study, the PCOS group was found to show worse performance in accurately recognising negative emotional expressions of fear and sadness compared with the control group, which may be explained by the finding of a correlation between high testosterone levels and poorer recognition accuracy of fearful faces. Detecting emotional cues and processing negative facial emotional expressions, such as fear and sadness, reflect an empathic response and emotional sensitivity, which may be negatively affected by high testosterone levels in the PCOS group. This may explain reduced accuracy in recognising threatening emotional expressions including fear, consistent with the findings of the current study, however, this does not justify findings related to decreased accuracy in recognising sad (non-threatening) facial expressions in the PCOS group.
To summarise, in the current study, the PCOS group showed worse recognition accuracy related to fearful and sad faces compared with the control group. Correlational analysis after controlling for mood showed a significant negative correlation only between FAI levels and recognition accuracy of fearful faces suggesting that higher FAI levels were associated with reduced sensitivity toward threatening faces which may be observed through worse recognition accuracy of fearful faces in the entire sample with varying levels of testosterone. It is possible that since the PCOS group experienced more symptoms of depression compared with the control group, these symptoms mediated the relationship between worse performance on measures of emotion processing and testosterone levels. Facial emotion processing deficits have been demonstrated in depression, including reduced accuracy of in recognising facial emotions of sadness, happiness and disgust in individuals with depression (Bediou et al., 2012; Bediou et al., 2009; Bourke et al., 2010; Douglas & Porter, 2010; Gotlib, Krasnoperova, et al., 2004; Leppänen, 2006; Leppänen et al., 2004; Leyman, De Raedt, Schacht, & Koster, 2007; Stuhrmann et al., 2011; Suslow et al., 2004). Future research specifically assessing the association between testosterone levels, mood and facial recognition involving fearful faces would be helpful to further examine this finding in detail.
7.14.1.3.2 Reading the Mind in the Eyes Test

In the current study, the RMET score did not significantly correlate with androgen levels (all \( p > 0.05 \)). However, significant group differences were observed, with the PCOS group showing worse performance on the RMET compared with the control group, after controlling for NART.

Previous studies examining the organisational effects of testosterone have found that high exposure to foetal testosterone may be negatively associated with empathic ability (see Chapter 3, Section 3.4.3). Females have been shown to have a significant advantage in empathic ability compared with males (E. Chapman et al., 2006). Additionally, as noted in Chapter Three, elevated foetal testosterone levels have been found in individuals with autism. Individuals with autism have also been shown to experience difficulties in empathy, which has been recently been proposed as a broader neurocognitive construct (Baron-Cohen, 2002; Sucksmith, Allison, Baron-Cohen, Chakrabarti, & Hoekstra, 2013). In line with this, one study examining organisational effects of testosterone found females with abnormally high levels of testosterone \textit{in utero} to show a higher number of autistic traits (including a lower empathic ability) compared with unaffected siblings (Knickmeyer et al., 2006), and another study found females with autism to have higher Free Testosterone levels compared with controls also suggesting the organisational effects of testosterone (Bejerot et al., 2012). However, since there are complications in investigating the organisational effects of pre-birth measures of testosterone, little research is found in this area. No cross-sectional study has used the RMET, so far, to assess emotion processing in females with PCOS. More research has been done using the RMET in interventional studies involving testosterone administration in healthy, reproductive-aged females, which will be discussed in Chapter Eight.
7.14.1.4 Associations between mood and cognitive function: Correlation analyses

In the current study, significant correlations were found between mood variables and some aspects of cognitive function, which is somewhat surprising since the sample in the current study experienced symptoms of depression of only mild-moderate severity. Thus, only a weak association with cognitive outcomes was expected. Analysis examining the association between mood and cognitive variables showed higher symptoms of depression to be significantly associated with poorer performance on the CVC Trial 1 and Digit Span variables, both of which measure verbal working memory. Additionally, greater symptoms of anxiety were significantly related to poorer performance on visuospatial learning and memory (errors on GMLT Trial 5).

Taken together, these results suggest that higher levels of depression and anxiety are associated with worse performance on measures assessing aspects of verbal working memory, executive function and attention, and spatial learning and memory. Previous studies have shown significant cognitive deficits in learning and memory in individuals with MDD compared with healthy controls (Austin et al., 2001; Douglas et al., 2013; Golinkoff & Sweeney, 1989; Sarosi et al., 2008; Thomas et al., 2009). In the current study, although aspects of verbal learning and memory and attention and executive function were found to be significantly negatively associated with mood, no other cognitive domains were found to be significantly associated with mood variables. However, it is likely that these could be chance findings.
Table 7.24  
*Summarising Correlations between Testosterone Variables and Mood and Anxiety Variables and Cognitive Function and Emotion Processing Variables across the Entire Sample (n = 103)*

<table>
<thead>
<tr>
<th></th>
<th>Free Androgen Index</th>
<th>Free Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS-D</td>
<td>0.32**</td>
<td></td>
</tr>
<tr>
<td>HADS Total Score</td>
<td>0.21*</td>
<td></td>
</tr>
<tr>
<td>CVC Recognition Accuracy</td>
<td>-0.206*</td>
<td>-0.233*</td>
</tr>
<tr>
<td>GMLT Total Errors Trial 5</td>
<td>-0.246*</td>
<td></td>
</tr>
<tr>
<td>GMLT Total Errors Trials 1-5</td>
<td>-0.228*</td>
<td></td>
</tr>
<tr>
<td>TCT (no of correct moves)</td>
<td>-0.262**</td>
<td>-0.310*</td>
</tr>
<tr>
<td>Recognition accuracy-fearful faces</td>
<td>-0.07*</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Positive correlational values suggest an association between higher levels of depression and higher levels of testosterone, and negative correlational values suggest an association between worse cognitive function and higher levels of testosterone. **= p < 0.01, *= p < 0.05.

**HADS-D** = Hospital Anxiety and Depression Scale (Depression sub-scale), **CVC** - Consonant Vowel Consonant Task, **GMLT** - Groton Maze Learning Test, **TCT** - Timed Chase Test

### 7.15 STRENGTHS OF THE STUDY

Previous findings related to the association between testosterone levels in females and mood and cognitive function are inconsistent, due to several factors. These include the inclusion of female samples with a broad age-range (including post-menopausal females), the inclusion of measures not suitable or adequately sensitive to detect the symptoms of depression unrelated to medical illness, small sample sizes, and an assessment of Total Testosterone but not Free Testosterone (unbound to plasma and the most important measure) or FAI levels. Strengths of the current study were: an inclusion of reproductive-aged females with PCOS together with a non-PCOS comparison group, and an inclusion of patients with varied physical and biochemical symptom profile seen through the wide spread of androgen levels. Another strength of the study was the use of a comprehensive mood and cognitive testing battery, including tasks assessing
emotion processing variables. Inclusion of the HADS (more suitable for assessing symptoms of depression and anxiety in a medical group such as PCOS) together with the interviewer-rated scale QIDS was a major strength of the current study. Importantly, this is the first known study to simultaneously investigate the relationship between androgen levels, mood, anxiety, cognitive function and emotion processing in females with PCOS.

7.15.1 Critique of the Research

7.15.1.1 Study design

The current study was a naturalistic longitudinal study in that the treatment for PCOS was not altered from the standard clinical treatment patients would generally receive at the gynaecological endocrine clinic, as a result of their participation in the current research study. Recruitment of patients with PCOS was more challenging than anticipated. The two main pathways to recruit females with PCOS were 1) referrals to gynaecological endocrine clinics, and 2) females responding to advertisements in local newspapers, following which they were assessed by the same treating Gynaecological-Endocrinologist (see Chapter Six, section 6.3.1). However, most patients with PCOS were recruited from clinics ($n = 33$), and thus, are likely to be representative of the population attending such clinics.

Symptoms observed in PCOS patients were heterogeneous, for example, hyperandrogenism observed through symptoms of acne, hirsutism and alopecia, together with higher BMI values and metabolic complications observed in the syndrome. Controlling for BMI was, therefore, not thought to be useful since it is a symptom of the complex syndrome rather than a separate factor that needs to be controlled for (see Chapter 6, section 6.11.2.1).
7.15.1.2 Medications at baseline

A limitation of the current study was the heterogeneity of anti-androgen and other hormonal medication used by patients. At baseline, a large proportion of patients reported using the OCP or COCP (46%) and four percent of the group reported using anti-androgen medication (see Table 7.5). Therefore, not all patients were unexposed to prior hormonal treatment which is a limitation of the current study. However, 86% of females were unexposed to anti-androgen medication (and Metformin) at baseline. Some patients had been taking medication for a significant period of time before changing to a different medication, or changing the dose, after being recruited into the current study. There was considerable heterogeneity in the types and doses of hormonal medication at baseline and also over the period of the study. This meant it was not possible to explore the associations between mood and cognitive tests and specific hormonal agents. However, the study also included a proportion of patients who attended the clinic for a medication review, in which case the dose or type of treatment was altered, compared with receiving treatment for the first time. This indicates that the current sample was representative of the general PCOS population.

7.15.1.3 Control group and sampling

The main reason for recruiting a control group in the current study was to have a group without PCOS which would generally have lower levels of androgens and would, therefore, provide a spread of androgen levels in the correlation analysis. Additionally, the inclusion of a non-PCOS comparison group was a strength of the current study, as practice effects in cognitive testing were able to be controlled for. However, this group was not well-matched with the PCOS group in terms of total years of education, verbal IQ and BMI, which was a significant limitation of the current study. Optimally, the control group should differ from the PCOS group only in the presence of PCOS. Current depression or a history of depression was not an exclusion for the control group because we intended to recruit a group which was not ‘supernormal’. This was a
convenience group which was characterised by being the same age as the PCOS group but not having a diagnosis of PCOS. Most control participants did not endorse symptoms of depression, however, some participants in this group showed mild symptoms of depression, assessed by the HADS (see Table 7.3). Due to sampling bias, females in the control group had higher verbal IQ scores compared with the PCOS group (see Section 7.15.1.6). This was a significant weakness and required statistical correction. Recruitment of control participants was intended to be more representative of the general population, and therefore, we tried to recruit from online resources, shopping malls, gymnasiums, and sports centres, however, a higher number of females from a university setting responded to advertisements regarding participation in the current study. This will have contributed to significant differences between the control group and the PCOS group on verbal IQ (NART) at baseline. A better strategy would have been to institute methods of recruiting controls which attracted those with lower IQs. Ideally, a comparison group should be sampled randomly from the same population as the illness (PCOS) group. Participants from the control group were not randomly selected. Therefore, it is not clear, for example, that depression rates are higher in PCOS than in a matched population without PCOS. People with depression may have been, indeed, less likely to volunteer.

7.15.1.4 Cognitive assessment

An issue which could be considered a limitation of the current study was the lack of inclusion of tasks assessing visuospatial ability which have been consistently shown to be related to testosterone levels in testosterone administration studies. However, we opted to focus on tasks which have previously also been shown to have lower levels of performance in depression. The cognitive testing battery contained a variety of measures including traditional cognitive tasks and emotion processing tasks, many of which have not been used in PCOS samples. The CVC Task assesses verbal learning and memory and presents nonsense syllables rather than presenting words. This task appears to be more sensitive to deficits in verbal learning and
memory in MDD compared with other tasks such as the RAVLT (Bourke et al., 2012; Douglas et al., 2011), and therefore, inclusion of this task added strength to the current study. However, the CVC is not a well-validated task.

7.15.1.5 The 2D:4D ratio

A putative marker of foetal testosterone levels is the digit length ratio, also known as the 2D:4D ratio (see Chapter 3, Section 3.4.3). Since the study included a thorough assessment of hormonal levels (particularly testosterone levels) and other physical symptoms of PCOS, collecting information about the digit length ratio was not considered important. However, this could possibly have added information regarding the interaction between organisational and activational effects of androgens. Future research examining emotion processing and cognitive function in females with high androgen levels may be benefitted by including this measure to examine both organisational and activational effects of testosterone together with their interaction.

7.15.1.6 Power/Sample size

The current study (including 50 patients and 53 controls) had 80% power to show a difference between the groups with an effect size of 0.6 (or above) as statistically significant, (two-tailed $\alpha=0.05$). Therefore, if effect sizes obtained in the current results were smaller than 0.6, the study was not adequately powered to show a statistically significant difference. Previous studies examining cognitive function in relation to testosterone levels in females with and without PCOS have tended to have smaller sample sizes (often less than 30 participants in the PCOS group). In the current study, recruitment was less than expected, since at the beginning of the study, the Canterbury District health Board (CDHB) published new “pathways” for General Practitioners mandating that the first-line treatment for patients with PCOS should involve OCPs/COCPs, following which referrals would be considered, which affected the sample size included in the
study. Although recruiting a large number of patients with PCOS was a priority for the current study, a major strength of the current study was the longitudinal study design involving anti-androgen treatment (discussed in Chapter 8, section 8.8).

7.15.1.7 Multiple testing

The issue of multiple outcome variables is important to consider. Given the exploratory nature of the study we opted not to utilise a statistical correction (for example, Bonferroni) for the number of statistical tests carried out. We opted instead to determine whether findings fitted a pattern and fitted with the literature. Thus, some findings may be likely to be attributed to chance (see Chapter 6, Section 6.11.1.1). It is important in the context of the current study to consider the possibility that the testing of multiple outcome variables may lead to the possibility of Type 1 errors, which means that statistically significant results could be chance findings. While acknowledging that many outcomes have been tested in this PCOS study, many of the outcomes are tested to confirm previous study results and additionally, individual statistically significant results are not discussed unless they fit into a coherent pattern with other significant results. The exploratory nature of much of the testing of associations is also acknowledged and significant results will need to be confirmed in future studies.

7.16 CONCLUSIONS

In the current study, symptoms of depression in the PCOS group were found to be significantly greater than those in the control group. This finding could be due to the high FAI levels found in females with PCOS. Indeed, this hypothesis was supported by the finding including a significant positive correlation between high FAI levels and greater symptoms of depression across the entire sample. Thus, this study provides evidence for a significant association between higher testosterone levels, specifically FAI levels, and mood in that higher FAI levels were found to be associated with greater depressive symptomatology.
Although the PCOS group did not perform significantly worse compared with the control group on most cognitive domains (except for psychomotor speed and emotion processing variables including recognition accuracy of fearful and sad faces, and the RMET), significant negative correlations were obtained between Free Testosterone and/or FAI levels and verbal learning and memory, visuospatial learning and memory, psychomotor speed and recognition accuracy (emotion processing) variables (see Table 7.24 for a summary of findings). These findings suggest that higher testosterone levels were associated with worse cognitive performance on measures of verbal learning and memory, spatial learning and memory, and psychomotor speed. However, multivariate analysis excluding BMI as a variable showed no significant associations between cognitive variables and Free Testosterone and/or FAI levels with the exception of a significant negative association between Free Testosterone levels and performance on the TCT (number of correct moves). Aspects of emotion processing were found to be impaired in the PCOS group compared with the control group. Testosterone levels were found to be significantly negatively correlated with emotion processing task variables. High levels of testosterone were associated with worse accuracy in recognising fearful facial expressions. Additionally, higher symptoms of depression were found to be significantly negatively associated with worse performance on verbal learning and memory and attention and executive function variables across the entire sample.
8.1 INTRODUCTION

Chapter Seven presented baseline findings regarding symptoms of depression and anxiety, and cognitive function and emotion processing in females with PCOS compared with the control group and correlations between testosterone levels and symptoms of mood, cognitive function and emotion processing. The focus of the current chapter is whether symptoms of mood, anxiety, cognitive function and emotion processing change following anti-androgen treatment in the PCOS group compared with the non-PCOS control group receiving no treatment, and whether there is an association between change in mood and anxiety and change in cognitive function and emotion processing in the PCOS group following anti-androgen treatment.

8.1.1 Aims

Major aims of the current chapter are:

- To compare the change between the PCOS group receiving treatment and the control group (non-PCOS comparison group not receiving treatment) on measures of mood, anxiety, cognitive function and emotion processing from the baseline to the follow-up (12-weeks after baseline) assessment.

- To examine change within the PCOS group following anti-androgen treatment on measures of mood, anxiety, cognitive function and emotion processing.
To determine whether there is an association between change in symptoms of mood and anxiety and change in cognitive function and emotion processing in the PCOS group following anti-androgen treatment.

8.1.2 General hypotheses

Until now, no known study has simultaneously investigated mood, cognitive function and emotion processing in females with PCOS. Furthermore, there has not been a concurrent examination of changes or improvements in symptoms of depression, anxiety, cognitive functioning and emotion processing over the course of anti-androgen treatment in females with PCOS. The current study hypothesised that:

- Anti-androgen treatment in the PCOS group will be associated with improvement in symptoms of depression and anxiety.
- Anti-androgen treatment in the PCOS group will be associated with improvement in cognitive function and emotion processing,
- Improvement in symptoms of depression and anxiety will be associated with improvement in cognitive function and emotion processing in the PCOS group over twelve-weeks of anti-androgen treatment.

8.1.3 Chapter outline

The first part of this chapter will present a description of participants included in the longitudinal study. Following this, changes in symptoms of mood, anxiety, cognitive function and emotion processing measures over the course of anti-androgen treatment in the PCOS group compared with the control group will be presented. Additionally, changes within the PCOS group on measures of mood, anxiety, cognitive function and emotion processing from the baseline to the follow-up assessment will be presented. Cognitive findings will be categorised into five main domains: verbal learning and memory, visuospatial (non-verbal) learning and
memory, attention and executive functioning, psychomotor speed and emotion processing. The remainder of this chapter will discuss correlational analyses examining associations between change in mood, anxiety and change in cognitive function and emotion processing following anti-androgen treatment in the PCOS group.

8.2 PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME

Not all females with PCOS completed the current longitudinal study. Fifty patients completed the initial baseline assessment. Eight patients did not complete the follow-up assessment at the twelve-week time-point due to relocating away from Christchurch (n = 3), being away for work (n = 1), and non-compliance with medication (did not start medication after baseline cognitive assessment) (n = 4). Thus, 42 patients with PCOS completed all assessments, a retention rate of 82%. Of the 53 control participants included at baseline, three did not complete the second assessment due to relocating away from Christchurch (n = 2) and starting anti-androgen treatment as a cosmetic measure for treating acne (n = 1). Therefore, the final sample consisted of 92 participants in all, including 42 patients with PCOS and 50 control participants.

Study completers (n = 42) were comparable to non-completers (n = 8) in the PCOS group on all demographic and clinical scales (see Table 8.1). The number of non-completers was small making statistical significance of any difference unlikely, and further examination of the data did not suggest any major group differences.

In the control group, 78% reported being in the luteal phase of the menstrual cycle while 22% reported being in the follicular phase at baseline. At the follow-up assessment, 84% of females were in the luteal phase, which was not significantly different to the proportion at baseline ($\chi^2[2] = 0.12, p = 0.52$).
Table 8.1

Means (SD) for Demographic Variables in Study Completers (n = 42) Versus Non-Completers (n = 8) in the PCOS group (n = 50)

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Completers</th>
<th>Non-completers</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.74</td>
<td>30.13</td>
<td>-0.44</td>
<td>0.67</td>
</tr>
<tr>
<td>BMI</td>
<td>27.42</td>
<td>29.84</td>
<td>-0.83</td>
<td>0.42</td>
</tr>
<tr>
<td>Predicted Verbal IQ (NART)</td>
<td>105.21</td>
<td>105.75</td>
<td>-0.14</td>
<td>0.89</td>
</tr>
<tr>
<td>Total years of education</td>
<td>8.36</td>
<td>7.00</td>
<td>0.93</td>
<td>0.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Completers</th>
<th>Non-completers</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline HADS</td>
<td>12.33</td>
<td>15.00</td>
<td>-0.83</td>
<td>0.93</td>
</tr>
<tr>
<td>Baseline HADS-D</td>
<td>4.71</td>
<td>5.75</td>
<td>-0.61</td>
<td>0.56</td>
</tr>
<tr>
<td>Baseline HADS-A</td>
<td>7.62</td>
<td>9.25</td>
<td>-0.92</td>
<td>0.38</td>
</tr>
<tr>
<td>Baseline QIDS</td>
<td>5.24</td>
<td>9.25</td>
<td>-1.87</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Androgen measures</th>
<th>Completers</th>
<th>Non-completers</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Testosterone</td>
<td>2.05</td>
<td>1.99</td>
<td>0.34</td>
<td>0.74</td>
</tr>
<tr>
<td>Free Testosterone</td>
<td>30.24</td>
<td>31.28</td>
<td>-0.20</td>
<td>0.84</td>
</tr>
<tr>
<td>Free Androgen Index</td>
<td>54.55</td>
<td>53.28</td>
<td>0.08</td>
<td>0.93</td>
</tr>
</tbody>
</table>

* t - Independent samples t-test, HADS- Hospital Anxiety and Depression Scale, HADS-A- Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D- Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS- Quick Inventory of Depressive Symptomatology, NART- National Adult Reading Test.
8.2.1 Anti-androgen treatment profile of patients with Polycystic Ovarian Syndrome

After baseline assessment, patients with PCOS were prescribed Metformin \((n = 6)\), CPA \((n = 9)\), Spironolactone \((n = 25)\), Flutamide \((n = 1)\), and/or the OCP/COCP \((n = 18)\). No patients with PCOS reported using antidepressant medication at the follow-up assessment.

Nine control participants reported using the OCP as a birth control measure at baseline which did not change during the study period. Females in the control group also reported using antidepressant medication (Fluoxetine, \(n = 1\); Escitalopram, \(n = 1\); Citalopram, \(n = 2\); Venlafaxine, \(n = 2\)), anti-psychotic medication prescribed in a low dose for insomnia (Quetiapine, \((n = 1)\) (100 mg) and anxiety medication (Lorazepam, \(n = 1\)). One female in the control group reported using minoxidil (antihypertensive vasodilator medication) as part of treatment for alopecia during the second assessment.

Table 8.2

Hormonal Treatment Profile of Patients with Polycystic Ovarian Syndrome at Follow-up

<table>
<thead>
<tr>
<th>Hormonal medication</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPA</td>
<td>9</td>
<td>(3.78)</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>25</td>
<td>(10.5)</td>
</tr>
<tr>
<td>Metformin</td>
<td>6</td>
<td>(2.52)</td>
</tr>
<tr>
<td>OCP or COCP (Yasmin, Ginette)</td>
<td>18</td>
<td>(7.56)</td>
</tr>
</tbody>
</table>

CPA - Cyproterone Acetate, COCP = combined oral contraceptive pill, IUD = Intra uterine device, OCP = oral contraceptive pill.
8.3 CHANGE IN SYMPTOMS OF DEPRESSION AND ANXIETY OVER THE COURSE OF TREATMENT IN THE POLYCYSTIC OVARIAN SYNDROME (n = 42) AND CONTROL (n = 50) GROUPS

An independent samples t-test was conducted to compare change in symptoms of depression and anxiety over 12 weeks in the PCOS group receiving treatment compared with the control group (not exposed to treatment). No significant differences were found between the two groups on change in symptoms of depression (HADS) over time. The PCOS group showed a reduction in total HADS score ($t = -1.84, p = 0.07$) and score on the depression subscale of the HADS (HADS-D) ($t = -1.90, p = 0.06$), however, at trend level, compared with the control group. No significant differences were found on change in the HADS-A (anxiety subscale) and the QIDS total score.

A paired t-test was conducted to test the change within the PCOS group on symptoms of depression and anxiety following 12 weeks of treatment. Scores on all scales assessing depression and anxiety showed significant improvement over the twelve-week study (see Table 8.3).

| Table 8.3 |
| Change in Symptoms of Depression and Anxiety Over the Course of Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups |

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th></th>
<th>Control</th>
<th></th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HADS total score</td>
<td>2.78**</td>
<td>5.74</td>
<td>0.72</td>
<td>4.85</td>
<td>-1.84</td>
<td>0.07</td>
<td>0.38</td>
</tr>
<tr>
<td>HADS-A</td>
<td>1.11*</td>
<td>2.76</td>
<td>0.30</td>
<td>2.87</td>
<td>-1.39</td>
<td>0.17</td>
<td>0.28</td>
</tr>
<tr>
<td>HADS-D</td>
<td>1.69**</td>
<td>3.55</td>
<td>0.42</td>
<td>2.69</td>
<td>-1.90</td>
<td>0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>QIDS total score</td>
<td>2.55***</td>
<td>4.31</td>
<td>1.28</td>
<td>3.92</td>
<td>-1.46</td>
<td>0.15</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*$t$ -Independent samples t-test, $d$ = Cohen’s $d$ effect size, $\text{HADS}$ = Hospital Anxiety and Depression Rating Scale, $\text{HADS-A}$ = Hospital Anxiety and Depression Scale (Anxiety sub-scale), $\text{HADS-D}$ = Hospital Anxiety and Depression Scale (Depression sub-scale), $\text{QIDS}$ = Quick Inventory of Depression Symptoms, $\text{PCOS}$ = Polycystic Ovarian Syndrome, *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, Asterisks values alongside the mean in the PCOS group represent significant change within the PCOS group from baseline to follow-up. Positive values denote improvement in symptoms of mood and anxiety.
8.4 CHANGE IN COGNITIVE FUNCTION OVER THE COURSE OF TREATMENT FOR POLYCYSTIC OVARIAN SYNDROME

Independent samples t-tests were conducted for all cognitive and emotion processing variables to determine whether there were any significant differences between the PCOS and control groups on change in cognitive functioning over the course of treatment. Effect sizes were calculated for the differences in change between the PCOS and control group from baseline to twelve-weeks (follow-up) for all cognitive measures.

8.4.1 Verbal learning and memory

8.4.1.1 Consonant-Vowel-Consonant Task

Differences in the change scores on CVC variables including Trial 1, Trial 5, Total Learning, Delayed Recall Trial, Recognition accuracy on List A were not significant between the PCOS and the control groups. However, significant improvements were found within the PCOS group on CVC variables (Trial 1, Trial 5, CVC Total learning, Delayed Recall) (all \(p < 0.001\)), with the exception of the recognition accuracy (see Table 8.4).

8.4.2 Visuospatial learning and memory

8.4.2.1 Groton Maze Learning Test

Differences in change on the GMLT variables including Total Errors on Trial 1, Trial 5, Trials 1-5, and on the Delayed Recall Trial were not significant between the PCOS and the control groups. However, within the PCOS group, there was a significant improvement on Trial 1 (\(p < 0.05\)), Trials 1-5 (\(p < 0.01\)) and the Delayed Trial (\(p < 0.001\)) of the GMLT (see Table 8.5).
Table 8.4
Means (SD) and Effect Sizes of Change in Consonant-Vowel-Consonant Task Variables following Anti-androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>t</td>
<td>p</td>
<td>d</td>
</tr>
<tr>
<td>CVC Trial 1</td>
<td>1.47***</td>
<td>1.90</td>
<td>1.28</td>
<td>1.90</td>
<td>0.49</td>
<td>0.62</td>
<td>0.10h</td>
</tr>
<tr>
<td>CVC Trial 5</td>
<td>2.21***</td>
<td>3.08</td>
<td>1.72</td>
<td>2.35</td>
<td>0.85</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td>CVC Total Learning</td>
<td>11.59***</td>
<td>8.35</td>
<td>8.68</td>
<td>8.53</td>
<td>1.65</td>
<td>0.10</td>
<td>0.34</td>
</tr>
<tr>
<td>Delayed Recall trial</td>
<td>2.26***</td>
<td>2.76</td>
<td>2.22</td>
<td>2.59</td>
<td>0.07</td>
<td>0.94</td>
<td>0.01</td>
</tr>
<tr>
<td>Recognition accuracy</td>
<td>0.00</td>
<td>2.43</td>
<td>-0.12</td>
<td>1.24</td>
<td>0.289</td>
<td>0.77</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* = p < 0.05, ** = p < 0.01, *** = p < 0.001, Asterisks values alongside the mean represent significant change within the PCOS group from baseline to follow-up. Positive values denote improvement in performance on the CVC Task over time.

\[ t \] = Independent samples t-test, \( d \) = Cohen’s d effect size, CVC = Consonant Vowel Consonant Task, PCOS = Polycystic Ovarian Syndrome.

Figure 8.1
Change in scores displayed as mean (+SD) number of words recalled on Trial 1, 5, 1-5 (total learning) and Delayed Recall Trial of the Consonant Vowel Consonant Task in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time.
Table 8.5  
*Means (SD) and Effect Sizes of Change in Groton Maze Learning Test Variables Following Anti-Androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>GMLT Total Errors</td>
<td>1.50*</td>
<td>3.98</td>
<td>0.46</td>
<td>4.41</td>
<td>-1.18</td>
</tr>
<tr>
<td>Trial 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMLT Total Errors</td>
<td>0.38</td>
<td>4.13</td>
<td>0.10</td>
<td>3.25</td>
<td>-0.35</td>
</tr>
<tr>
<td>Trial 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMLT Total Errors</td>
<td>4.83**</td>
<td>10.70</td>
<td>1.68</td>
<td>13.81</td>
<td>-1.23</td>
</tr>
<tr>
<td>Trials 1-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMLT Delayed Trial</td>
<td>2.26***</td>
<td>2.75</td>
<td>2.22</td>
<td>2.58</td>
<td>-0.75</td>
</tr>
<tr>
<td>(Trial 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.94</td>
</tr>
</tbody>
</table>

*T = Independent samples t-test, d = Cohen’s d effect size, GMLT = Groton Maze Learning Test, PCOS = Polycystic Ovarian Syndrome, * = p < 0.05, ** = p < 0.01, *** = p < 0.001, Asterisks’ values alongside the mean represent significant change within the PCOS group from baseline to follow-up. Positive values denote improvement in performance on the spatial learning and memory task.*

**Figure 8.2:**  
Change in scores displayed as mean (± SD) total number of errors on the Groton Maze Learning Test variables in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time.
8.4.3 Psychomotor Speed

8.4.3.1 Timed Chase Test

Significant differences were found between the PCOS and control groups on change in performance on the TCT (number of correct moves: \( t = 3.19, p < 0.01 \); total number of errors: \( t = -2.55, p < 0.01 \)). The difference between groups on performance on the TCT over time was found to be significant, with the PCOS group showing better performance compared with the control group at the follow-up assessment. The control group was found to show worse performance over time. No significant change was observed within the PCOS group following anti-androgen treatment on the TCT.

8.4.3.2 Trail Making Test-Part A

With regards to the TMT- Part A (time taken), no significant differences in change were found between the two groups over time. Additionally, no significant change was observed within the PCOS group from baseline to follow-up on this task.

Table 8.6

Means (SD) and Effect Sizes of Change in Psychomotor Speed Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCT (no of Correct moves)</td>
<td>1.59 (7.40)</td>
<td>-4.06 (9.56)</td>
<td>3.19</td>
<td>0.002</td>
<td>0.66</td>
</tr>
<tr>
<td>TCT (total errors)</td>
<td>0.31 (1.38)</td>
<td>0.76 (2.54)</td>
<td>-2.55</td>
<td>0.01</td>
<td>0.52</td>
</tr>
<tr>
<td>TMT – A (seconds)</td>
<td>6.16 (20.28)</td>
<td>5.40 (15.33)</td>
<td>-0.20</td>
<td>0.84</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\( t \) = Independent samples \( t \)-test, \( d \) = Cohen’s \( d \) effect size, \( * = p < 0.05 \), \( ** = p < 0.01 \), \( *** = p < 0.001 \), TCT = Timed Chase Test, TMT = Trail Making Test, PCOS = Polycystic Ovarian Syndrome. Positive values represent improvement on the task, whereas negative values represent worse performance.
8.4.4 Attention and executive functioning

No significant differences were found between PCOS \((n = 42)\) and control \((n = 50)\) groups, or within the PCOS group, on any of the attention and executive function variables (TMT–Part B, COWAT, Digit Span subtests) over time (see Table 8.7).

8.5 CHANGE IN EMOTION PROCESSING OVER THE COURSE OF TREATMENT FOR POLYCYSTIC OVARIAN SYNDROME

8.5.1 Facial Expression Recognition Task

8.5.1.1 Recognition accuracy

No significant differences in change in recognition accuracy were observed between the PCOS \((n = 42)\) and control \((n = 50)\) groups over time for any facial expressions of emotion. However, a significant improvement in recognising disgusted facial expressions was observed within the PCOS group \((p < 0.05)\) following anti-androgen treatment (see Table 8.8).

8.5.1.2 Reaction time

A significant difference was observed between the PCOS \((n = 42)\) and control \((n = 50)\) groups on change in reaction time to faces depicting fear over time \((t = -2.21, p = 0.03)\). Significant improvement was found on change in reaction time for all facial expressions, with the exception of happy faces, within the PCOS group following anti-androgen treatment (See Table 8.9).
Table 8.7

Means (SD) and Effect Sizes of Change in Attention and Executive Function Variables in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS</th>
<th>Control</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>TMT–B (seconds)</td>
<td>8.23</td>
<td>24.17</td>
<td>7.40</td>
<td>17.43</td>
<td>-0.20</td>
</tr>
<tr>
<td>COWAT (total words)</td>
<td>1.00</td>
<td>7.32</td>
<td>0.58</td>
<td>8.47</td>
<td>0.25</td>
</tr>
<tr>
<td>Digit Span Forward Total</td>
<td>-0.02</td>
<td>2.04</td>
<td>-0.14</td>
<td>1.54</td>
<td>0.30</td>
</tr>
<tr>
<td>Digit Span Forward Span</td>
<td>-0.14</td>
<td>1.11</td>
<td>-0.26</td>
<td>0.94</td>
<td>0.53</td>
</tr>
<tr>
<td>Digit Span Backward Total</td>
<td>0.52</td>
<td>2.16</td>
<td>0.50</td>
<td>2.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Digit Span Backward Span</td>
<td>0.19</td>
<td>1.35</td>
<td>0.34</td>
<td>1.27</td>
<td>-0.54</td>
</tr>
<tr>
<td>Digit Span Total Score</td>
<td>0.73</td>
<td>2.66</td>
<td>0.40</td>
<td>2.41</td>
<td>0.63</td>
</tr>
</tbody>
</table>

_t_ = Independent sample _t_-test, _d_ = Cohen’s _d_ effect size, TMT = Trail Making Test, COWAT = Controlled Oral Word Association Test, Positive values denote improvement in performance on attention and executive function variables
Table 8.8

*Means (SD) and Effect Sizes of Change in Recognition Accuracy on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Angry</td>
<td>0.00</td>
<td>0.13</td>
<td>0.01</td>
<td>0.13</td>
<td>-0.74</td>
</tr>
<tr>
<td>Disgusted</td>
<td>0.06*</td>
<td>0.16</td>
<td>0.05</td>
<td>0.19</td>
<td>0.36</td>
</tr>
<tr>
<td>Fearful</td>
<td>0.02</td>
<td>0.11</td>
<td>0.01</td>
<td>0.09</td>
<td>0.35</td>
</tr>
<tr>
<td>Happy</td>
<td>0.02</td>
<td>0.11</td>
<td>0.00</td>
<td>0.07</td>
<td>1.48</td>
</tr>
<tr>
<td>Neutral</td>
<td>0.00</td>
<td>0.16</td>
<td>0.02</td>
<td>0.10</td>
<td>-0.38</td>
</tr>
<tr>
<td>Sad</td>
<td>0.04</td>
<td>0.17</td>
<td>0.02</td>
<td>0.16</td>
<td>0.44</td>
</tr>
<tr>
<td>Total accuracy</td>
<td>0.02</td>
<td>0.05</td>
<td>0.02</td>
<td>0.67</td>
<td>0.20</td>
</tr>
</tbody>
</table>

$t =$ Independent sample $t$-test, $d =$ Cohen’s $d$ effect size, $PCOS =$ Polycystic Ovarian Syndrome, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Asterisks’ values alongside the mean represent significant change within the PCOS group following anti-androgen treatment. Positive mean values denote an improvement in recognition accuracy.

Figure 8.3

*Change in scores displayed as mean (±SD) recognition accuracy for the five facial expressions of emotion and neutral expressions on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time.*
Table 8.9
Means (SD) and Effect Sizes of Change in Reaction Time on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Angry</td>
<td>228.82**</td>
<td>447.56</td>
<td>170.85</td>
<td>764.38</td>
<td>-0.452</td>
</tr>
<tr>
<td>Disgusted</td>
<td>319.73***</td>
<td>384.00</td>
<td>183.23</td>
<td>636.46</td>
<td>-1.267</td>
</tr>
<tr>
<td>Fearful</td>
<td>270.45***</td>
<td>314.35</td>
<td>132.15</td>
<td>279.83</td>
<td>-2.209</td>
</tr>
<tr>
<td>Sad</td>
<td>155.69**</td>
<td>355.28</td>
<td>133.82</td>
<td>616.47</td>
<td>-0.212</td>
</tr>
<tr>
<td>Happy</td>
<td>73.73</td>
<td>279.76</td>
<td>14.33</td>
<td>299.93</td>
<td>-0.982</td>
</tr>
<tr>
<td>Neutral</td>
<td>206.88***</td>
<td>334.29</td>
<td>138.42</td>
<td>321.88</td>
<td>-0.995</td>
</tr>
<tr>
<td>Mean Reaction Time</td>
<td>208.75***</td>
<td>235.90</td>
<td>130.72</td>
<td>338.08</td>
<td>-1.298</td>
</tr>
</tbody>
</table>

*t* = Independent sample *t*-test, *d* = Cohen’s *d* effect size, * = *p* < 0.05, ** = *p* < 0.01, *** = *p* < 0.001. Positive mean values indicate an improvement in reaction time.

8.5.1.3 Neutral misinterpretation bias

A significant difference was found between groups on change in the misinterpretation of neutral facial expressions over time, with the PCOS group becoming significantly less likely to interpret neutral faces as angry over time compared with the control group (*t* = -2.69, *p* = 0.009).

Additionally, a significant change was found in the misinterpretation of neutral faces as angry within the PCOS group following anti-androgen treatment (see Table 8.10), with neutral faces being less likely to be interpreted as angry over time.
Table 8.10
Means (SD) and Effect Sizes of Change in Neutral Misinterpretation Bias on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>t</td>
<td>p</td>
</tr>
<tr>
<td>Anger</td>
<td>-0.07</td>
<td>0.28</td>
<td>-0.02</td>
<td>0.24</td>
<td>1.65</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>0.13*</td>
<td>0.32</td>
<td>0.02</td>
<td>0.21</td>
<td>-2.69</td>
<td>0.009**</td>
</tr>
<tr>
<td>Disgust</td>
<td>-0.00</td>
<td>0.09</td>
<td>-0.00</td>
<td>0.13</td>
<td>0.46</td>
<td>0.64</td>
</tr>
<tr>
<td>Happy</td>
<td>-0.02</td>
<td>0.15</td>
<td>-0.01</td>
<td>0.12</td>
<td>1.17</td>
<td>0.24</td>
</tr>
<tr>
<td>Sad</td>
<td>-0.03</td>
<td>0.18</td>
<td>0.01</td>
<td>0.18</td>
<td>0.47</td>
<td>0.63</td>
</tr>
</tbody>
</table>

- Independent sample t-test.
- * = p < 0.05,
- ** = p < 0.01,
- *** = p < 0.001.
- Asterisks’ values alongside the mean represent significant change within the PCOS group following anti-androgen treatment, positive mean values indicate that neutral faces were less likely to be interpreted as a particular emotion.

Figure 8.4
Change in scores displayed as mean (+SD) misinterpretation of neutral faces for the five facial expressions of emotion on the Facial Expression Recognition Task in the Polycystic Ovarian Syndrome (n = 42) and control (n = 50) groups over time.
8.5.2 Reading the Mind in the Eyes Test

There was no significant difference between the PCOS (0.45±3.30) and control (0.24±2.76) groups vs \( t = 0.33, \ p = 0.742 \) on change in RMET scores over time. Additionally, no significant change was observed within the PCOS group following anti-androgen treatment \( t = 0.463, \ p = 0.68 \).

8.6 CORRELATIONAL ANALYSES

The following section will present findings of correlations between change in mood and anxiety, and change in cognitive function and emotion processing in the PCOS sample \( n = 42 \). Since the control group did not receive treatment following baseline assessment, no significant change in mood, cognitive function or emotion processing performance was expected in this group over time, therefore, analyses comprised of correlations only within the PCOS group. Results from these analyses will be discussed in the next section.

8.6.1 Correlations between mood and anxiety variables and cognitive variables in the Polycystic Ovarian Syndrome Group

A bivariate correlational analysis was conducted to investigate the relationship between change in scores on mood and anxiety variables and change in cognitive variables in the PCOS group following treatment. Findings from this analysis are presented in the following sections.

8.6.1.1 Verbal learning and memory

No significant associations were found between change in mood and anxiety variables, and change in performance on CVC Task variables in the PCOS group following treatment (see Table 8.11).
Table 8.11

Correlations between Change in Mood and Anxiety Variables and Change in Consonant-Vowel-Consonant Task Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>CVC Trial 1</th>
<th>CVC Trial 5</th>
<th>CVC Trial 1-5</th>
<th>Delayed recall</th>
<th>Recognition accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total</td>
<td>0.14</td>
<td>0.03</td>
<td>-0.02</td>
<td>-0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.12</td>
<td>-0.02</td>
<td>-0.07</td>
<td>-0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.12</td>
<td>0.11</td>
<td>0.03</td>
<td>-0.00</td>
<td>-0.05</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.08</td>
<td>0.01</td>
<td>-0.04</td>
<td>-0.03</td>
<td>-0.10</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between changes in scores on mood/anxiety scales and changes in cognitive variables from baseline to follow-up). HADS = Hospital Anxiety and Depression Rating Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick Inventory of Depression Symptoms, CVC = Consonant Vowel Consonant Test. Positive correlations suggest an association between improvement in symptoms of depression and anxiety and improvement in verbal learning and memory.

8.6.1.2 Visuospatial learning and memory

No significant correlations were found between changes in mood and anxiety variables and changes in performance on GMLT Task variables (see Table 8.12).

8.6.1.3 Psychomotor speed

Significant positive correlations were found between change in performance on the TCT (number of correct moves) and change in mood and anxiety variables (HADS Total, r = 0.37, p = 0.01; HADS-A, r = 0.37, p = 0.01; QIDS, r = 0.40, p = 0.009, and HADS-D, r = -0.31, p = 0.04). This indicated that improvement in symptoms of depression and anxiety were associated with better performance on the TCT (more number of correct moves) assessing psychomotor speed (see Table 8.13). A significant positive correlation was found between change in performance on the TMT- Part A (total time taken) and change in the symptoms of depression (QIDS) (r = 0.33, p = 0.03), suggesting that an improvement in symptoms of depression was associated with better performance on the TMT - Part A.
Table 8.12

Correlations between Change in Mood and Anxiety Variables and Change in Groton Maze Learning Test Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>GMLT Errors Trial 1</th>
<th>GMLT Errors Trial 5</th>
<th>GMLT Errors Trials 1-5</th>
<th>GMLT Errors Delay Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total Score</td>
<td>0.09</td>
<td>-0.10</td>
<td>-0.08</td>
<td>-0.05</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.13</td>
<td>-0.17</td>
<td>-0.12</td>
<td>-0.07</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.03</td>
<td>-0.00</td>
<td>-0.01</td>
<td>-0.00</td>
</tr>
<tr>
<td>QIDS</td>
<td>-0.13</td>
<td>-0.04</td>
<td>-0.28</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between changes in scores on mood/anxiety variables and cognitive variables from baseline to follow-up). HADS = Hospital Anxiety and Depression Rating Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick Inventory of Depression Symptoms, GMLT- Groton Maze Learning Test, Positive correlations indicate that improvement in symptoms of depression was associated with improvement in GMLT performance (less errors).

8.6.1.4 Attention and executive function

A significant positive correlation was found between change in performance on the TMT- Part B (total time taken) and change in the symptoms of depression (QIDS) \( (r = 0.30, p = 0.05) \). Significant positive correlations were also found between change in Total Forward score (Digit Span) and change in symptoms of depression (HADS Total, \( r = 0.31, p = 0.04 \); and HADS-A, \( r = 0.32, p = 0.04 \)) and Forward Span score (Digit Span) and change in symptoms of depression (HADS Total, \( r = 0.30, p = 0.05 \); and HADS-A, \( r = 0.31, p = 0.04 \)). No other correlations were significant.
Table 8.13  
Correlations between Change in Mood and Anxiety Variables and Change in Psychomotor Speed Variables Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome (n = 42) and Control (n = 50) Groups

<table>
<thead>
<tr>
<th></th>
<th>TCT (no of correct moves)</th>
<th>TCT (errors)</th>
<th>TMT Part A (time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total Score</td>
<td>0.37**</td>
<td>0.16</td>
<td>0.28</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.31*</td>
<td>0.08</td>
<td>0.29</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.37**</td>
<td>0.23</td>
<td>0.19</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.40**</td>
<td>0.00</td>
<td>0.33*</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between changes in scores on mood/anxiety variables and cognitive variables from baseline to follow-up). HADS = Hospital Anxiety and Depression Rating Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick Inventory of Depression Symptoms, TCT = Timed Chase Test, TMT = Trail Making Task. * = p < 0.05, ** = p < 0.01, *** = p < 0.001. Positive correlations indicate improvement in symptoms of depression and anxiety and improvement in psychomotor speed over time.

8.6.2 Correlations between mood and anxiety variables and emotion processing variables in the Polycystic Ovarian Syndrome Group

The relationship between change in mood and anxiety variables and change on FER Task variables and RMET variables was examined using bivariate correlational analysis. These findings will be discussed in the following section.

8.6.2.1 Recognition accuracy (Facial Expression Recognition Task)

Significant positive correlations were found between change in recognition accuracy of fearful faces and change in HADS Total Score ($r = 0.64, p = 0.001$), HADS-D score ($r = 0.60, p = 0.002$) and HADS-A score ($r = 0.58, p = 0.007$), suggesting an association between improvement in symptoms of depression and anxiety and improvement in recognition accuracy of fearful faces. A significant positive correlation was found between change in symptoms of depression (HADS-D) and change in performance on recognition of the facial expression of disgust ($r = 0.32, p = 0.04$) on the FER Task. No other correlations were significant over time in the PCOS group.
Table 8.14
Correlations between Change in Mood and Anxiety Variables and Change in Attention and Executive Function Variables following Anti-Androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>TMT Part B (time)</th>
<th>COWAT Total Score</th>
<th>Digit Span Total Forward</th>
<th>Digit Span Forward Span</th>
<th>Digit Span Total Backward</th>
<th>Digit Span Backward Span</th>
<th>Digit Span Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total Score</td>
<td>0.27</td>
<td>-0.05</td>
<td>0.31*</td>
<td>0.30*</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.29</td>
<td>0.05</td>
<td>0.25</td>
<td>0.24</td>
<td>0.06</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.19</td>
<td>-0.18</td>
<td>0.32*</td>
<td>0.31*</td>
<td>-0.10</td>
<td>-0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>QIDS</td>
<td><strong>0.30</strong></td>
<td>-0.18</td>
<td>0.22</td>
<td>0.19</td>
<td>0.16</td>
<td>-0.19</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between changes in scores on mood/anxiety variables and cognitive variables from baseline to follow-up), ** = p < 0.01, * = p < 0.05. HADS - Hospital Anxiety and Depression Rating Scale, HADS-A - Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D - Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS - Quick Inventory of Depression Symptoms, TMT-Trail Making Test, COWAT-Controlled Oral Word Association Test, positive correlations indicate improvement in symptoms of depression and anxiety and improvement in performance on tasks of attention and executive function.
Table 8.15
Correlations between Change in Mood and Anxiety Variables and Accuracy on the Facial Expression Recognition Task Following Anti-Androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>Angry</th>
<th>Disgusted</th>
<th>Fearful</th>
<th>Happy</th>
<th>Neutral</th>
<th>Sad</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total</td>
<td>0.05</td>
<td>0.27</td>
<td><strong>0.64</strong>*</td>
<td>0.03</td>
<td>-0.21</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.04</td>
<td><strong>0.32</strong>*</td>
<td><strong>0.60</strong>*</td>
<td>0.01</td>
<td>-0.15</td>
<td>0.06</td>
<td>0.18</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.03</td>
<td>0.14</td>
<td><strong>0.58</strong>*</td>
<td>0.04</td>
<td>-0.26</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.00</td>
<td>0.13</td>
<td>0.22</td>
<td>0.21</td>
<td>-0.19</td>
<td>-0.07</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between the change in the score of mood/anxiety variables and cognitive variables from baseline to follow-up), * = p < 0.05, ** = p < 0.01, *** = p < 0.001. HADS = Hospital Anxiety and Depression Rating Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick Inventory of Depression Symptoms, positive correlations suggest that improvement in symptoms of mood and anxiety was associated with improvement in recognition accuracy on the FER Task.

8.6.2.2 Reaction time (Facial Expression Recognition Task)

No significant correlations were found between change in reaction time on the FER Task and change in mood or anxiety variables (see Table 8.16).

Table 8.16
Correlations between Change in Mood and Anxiety Variables and Mean Reaction Time on the Facial Expression Recognition Task Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>Angry</th>
<th>Disgusted</th>
<th>Fearful</th>
<th>Happy</th>
<th>Neutral</th>
<th>Sad</th>
<th>Mean RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total</td>
<td>0.00</td>
<td>0.10</td>
<td>0.04</td>
<td>-0.16</td>
<td>-0.11</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.02</td>
<td>0.16</td>
<td>0.03</td>
<td>-0.21</td>
<td>-0.05</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>HADS-A</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.06</td>
<td>-0.08</td>
<td>-0.18</td>
<td>-0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.13</td>
<td>0.15</td>
<td>0.03</td>
<td>0.00</td>
<td>0.09</td>
<td>-0.12</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between changes in scores on mood and cognitive variables from baseline to follow-up), HADS = Hospital Anxiety and Depression Rating Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick Inventory of Depression Symptoms.
Depression Symptoms, RT = reaction time, positive correlations suggest that improvement in symptoms of mood and anxiety was associated with improvement in reaction times on the FER Task.

8.6.2.3 Neutral misinterpretation bias (Facial Expression Recognition Task)

No correlations between change in mood and anxiety variables and change in neutral misinterpretation bias for each emotion were significant at the follow-up assessment (see Table 8.17).

Table 8.17
Correlations between Change in Mood and Anxiety Variables and Neutral Misinterpretation Bias on the Facial Expression Recognition Task Following Anti-androgen Treatment in the Polycystic Ovarian Syndrome Group (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>Angry</th>
<th>Disgusted</th>
<th>Fearful</th>
<th>Happy</th>
<th>Sad</th>
</tr>
</thead>
<tbody>
<tr>
<td>HADS Total</td>
<td>0.15</td>
<td>-0.06</td>
<td>0.13</td>
<td>-0.30</td>
<td>-0.01</td>
</tr>
<tr>
<td>HADS-D</td>
<td>0.23</td>
<td>-0.14</td>
<td>0.12</td>
<td>-0.26</td>
<td>-0.06</td>
</tr>
<tr>
<td>HADS-A</td>
<td>0.02</td>
<td>0.05</td>
<td>0.12</td>
<td>-0.29</td>
<td>0.05</td>
</tr>
<tr>
<td>QIDS</td>
<td>0.15</td>
<td>-0.09</td>
<td>-0.06</td>
<td>-0.14</td>
<td>-0.00</td>
</tr>
</tbody>
</table>

Pearson product-moment correlation (correlations are between changes in scores on mood/anxiety variables and cognitive variables from baseline to follow-up). HADS = Hospital Anxiety and Depression Rating Scale, HADS-A = Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D = Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS = Quick Inventory of Depression Symptoms, positive correlations suggest that improvement in symptoms of mood and anxiety was associated with reduced tendency to interpret neutral faces as expressing an emotion.

8.6.2.4 Reading the Mind in the Eyes Test

Change in the total RMET score was not significantly correlated with change in mood or anxiety variables in the PCOS group following anti-androgen treatment (all \( p > 0.10 \)).
8.7 DISCUSSION

The aims of this chapter were:

- To compare changes between the PCOS and the control groups on measures of mood, anxiety, cognitive function and emotion processing over time.
- To determine whether there was a change in symptoms of depression, anxiety, cognitive function and emotion processing within the PCOS group following anti-androgen treatment over a period of 12 weeks.
- To investigate the association between change in mood and anxiety variables and change in cognitive function and emotion processing variables in the PCOS group following anti-androgen treatment.

The remainder of this chapter will discuss the main findings in relation to these aims.

8.7.1 Main findings

There was a trend for symptoms of depression to improve in the PCOS group following anti-androgen treatment, compared with the control group receiving no treatment ($p < 0.06$).

Significant improvement was found on all scales assessing symptoms of depression and anxiety within the PCOS group following anti-androgen treatment. Since anxiety levels were not found to be significantly different between groups at baseline, no significant change was expected in the PCOS group in comparison with the control group at the follow-up assessment. In line with this hypothesis, no significant improvements were observed in symptoms of anxiety following anti-androgen treatment in the PCOS group compared with the control group.

The main results from correlational analyses were:

- Change in symptoms of depression and anxiety significantly positively correlated with change in performance on the TCT (number of correct moves), indicating that improved
mood and anxiety levels were associated with better performance on the TCT, a measure of psychomotor speed.

- Change in symptoms of depression (QIDS) significantly positively correlated with change in TMT - Part A (time), suggesting that improvement in mood symptoms was associated with improved performance on the TMT - Part A, a measure of psychomotor speed.

- Change in symptoms of depression (QIDS) significantly positively correlated with change in TMT - Part B, suggesting that improved mood was associated with improved performance on the TMT - Part B, assessing attention and executive function (specifically, cognitive flexibility).

- Change in symptoms of depression and anxiety (HADS) significantly positively correlated with change in the two Digit Span Forward variables (Total Forward and Forward span), assessing attention and executive function (specifically, working memory), suggesting that as symptoms of depression improved, performance on this measure of attention and executive function improved too.

- Change in symptoms of depression and anxiety (HADS) significantly positively correlated with change in recognition accuracy of fearful faces, suggesting that improvements in symptoms of depression and anxiety were associated with improved ability to recognise fearful facial expressions on the FER Task.

- Change in symptoms of depression (HADS-D) significantly positively correlated with change in recognition accuracy of disgusted facial expressions, suggesting that improved depression symptoms was associated with improved recognition accuracy of disgusted facial expressions on the FER Task.

At baseline, apart from a significant difference between the two groups on the TCT (ES = 0.76), no other differences were found between the two groups on any other cognitive variables, after controlling for NART scores. Therefore, a change in performance on the TCT would be an
expected cognitive outcome over time. In line with this hypothesis, over the study period, significant improvements were observed on change in this psychomotor speed variable. The PCOS group did not show significant improvements in performance on cognitive domains of verbal and visuospatial learning and memory and attention and executive function compared the control group.

Regarding emotion processing, at baseline, univariate ANOVA found the PCOS group to be significantly less accurate in recognising facial expressions of fear \((F = 1.93, p = 0.04)\) and sadness \((F = 5.97, p = 0.004)\) and to perform significantly worse compared with the control group on the RMET \((F = 8.14, p = 0.005)\) \((26.78 \pm 0.47 \text{ vs } 28.10 \pm 0.46; \text{ ES } = 0.41)\) compared with the control group (values expressed in percentages, see Table 7.11). However, at baseline, no significant differences were found between the two groups on facial emotion processing variables including reaction time and neutral misinterpretation bias on the FER Task. Therefore, changes in the former variables at follow-up would be more likely compared with other emotion processing variables. However, no change in recognition accuracy of fearful or sad faces was found in the PCOS group compared with the control group over time. Somewhat unexpectedly, reaction time related to fearful faces improved in the PCOS group compared with the control group over the study period. As noted previously testosterone has been associated with a decreased sensitivity to fearful or threatening faces (see Chapters Five and Seven). The finding of improved (decreased) reaction time related to fearful faces in the PCOS group suggests an association with decreased testosterone levels found post-treatment. Additionally, it could be that since the PCOS group improved on other measures of psychomotor speed, an improvement in reaction time simply reflects better overall psychomotor speed. The PCOS group was also found to improve in neutral misinterpretation bias of angry faces (reduced tendency to interpret neutral facial expressions as angry) compared with the control group over time. Research suggests that testosterone administration is associated with a reduction in sensitivity toward negative or threatening faces (particularly fearful and angry faces) observed through reduced
gaze aversion and gaze avoidance (a socially anxious pattern) and increased attention toward negative faces (see Chapters Five and Seven). These results would suggest that anti-androgen treatment may increase sensitivity toward threatening faces, making it more likely to increase neutral misinterpretation bias (worse performance), contrary to current results. However, testosterone administration studies may not necessarily show the opposite results of studies involving anti-androgen administration (see section 8.7.1.1 for further discussion).

Additionally, since the total score on RMET assessing “social intelligence” was found to be significantly different between the two groups at baseline, it was expected that anti-androgen treatment would affect this result at the follow-up assessment too. However, contrary to this hypothesis, no significant change in performance on RMET was found between the two groups, over time.

Within the PCOS group, significant changes were observed on some cognitive and emotion processing measures following anti-androgen treatment. These included changes/improvement on tasks assessing verbal learning and memory, visuospatial learning and memory and recognition accuracy, reaction time and neutral misinterpretation bias on the FER Task. However, since performance on these domains was not always significantly different compared with the control group, these changes within the group may, therefore, be attributed largely to practice effects. A healthy control group is necessary when there are repeated cognitive assessments to examine the degree to which change in cognitive performance is related to symptom change (including mood and testosterone levels changing) as opposed to practice effects from repeated task administration. Despite the use of parallel forms, improvement may occur due to enhanced learning strategies, and therefore, results showing significant change within the PCOS group must be understood keeping the issue of practice effects in mind. However, given the correlations with change in mood, it is also possible that anti-androgen treatment may have resulted in subtle overall improvements in mood and cognitive function.
within the PCOS group, which in comparison with changes in the control group, did not quite reach significance because of the small group sizes.

Effect sizes of the difference between change in cognitive function in the PCOS group compared with change in cognitive function in the control group were found to be small to moderate (0.0 to 0.4) with the exception of the TCT which was moderate (0.6). The next section will discuss findings related to mood, anxiety and cognitive variables in comparison with previous studies.

8.7.1.1 Findings related to mood and anxiety: group comparisons

Until now, most studies and reviews have examined mood in females with PCOS at only one time-point, without a longitudinal assessment of mood (see Chapter 5 for a review of literature), and have found variable results related to the association between androgen levels and mood in females of reproductive age. The current study used a longitudinal study design to examine the effect of anti-androgen treatment on change in symptoms of depression, anxiety, cognitive function and emotion processing in females with PCOS. Anti-androgen treatment with different mechanisms of action included agents such as CPA, Spironolactone, OCPs, COCPs, and other medication included Metformin (aiming to improve metabolic abnormalities found in PCOS) (see Chapter 5, Section 4.7.2.4). Anti-androgen medication aims to prevent androgens including testosterone and DHT from acting on receptors by either blocking the androgen receptor or by inhibiting or suppressing androgen production (Escobar-Morreale et al., 2011; Falsetti et al., 2000; Koulouri & Conway, 2008; Swiglo et al., 2008; Townsend & Marlowe, 2004; Van Zuuren & Fedorowicz, 2015; Venturoli et al., 1999; Yildiz, 2008). In the current study, CPA and Spironolactone were the main agents used in conjunction with the OCP (particularly in the case of sexually active females), as considered appropriate by the treating Gynaecological-Endocrinologist based on each individual patient’s symptom profile (refer to Chapter 7, Section 7.4.3, and Chapter 8, Section 8.2.2). CPA and Spironolactone show similar efficacy and are
androgen receptor antagonists that bind directly to and block the androgen receptor and reduce androgen biosynthesis (Erenus, Yucelten, Gurbuz, Durmusoglu, & Pekin, 1996; Menard, Stripp, & Gillette, 1974; Messina et al., 1983; Mowszowicz et al., 1984). Specifically, Spironolactone as an anti-androgen acts primarily at the periphery to inhibit 5-a-reductase activity, while CPA competitively inhibits the binding of testosterone and DHT to androgen receptors (Erenus et al., 1996). Spironolactone has the additional effect of preventing conversion of testosterone to DHT and CPA has a hypothalamic suppressive effect when used in moderate dose, continuously. Post-treatment testosterone levels may not necessarily indicate a decrease in testosterone levels but may work by blocking the androgen receptor. Since the current study included different treatment agents, measurement of post-treatment testosterone levels was not considered useful. Therefore, investigations only included correlations between changes in mood and anxiety and changes in cognitive function and emotion processing in the PCOS group following treatment and not correlations with testosterone levels.

At baseline, the PCOS group showed greater symptoms of depression than the control group. Following anti-androgen treatment, symptoms of depression and anxiety were still higher in the PCOS group compared with the control group, however, differences between groups at the follow-up assessment on mood and anxiety measures were not significant. Additionally, significant improvement in symptoms of depression and anxiety was observed within the PCOS group following anti-androgen treatment (see Table 8.3). Although previous studies have suggested an association between physical symptoms of PCOS such as hirsutism, acne, weight gain and infertility with symptoms of low mood and anxiety (Asik et al., 2015; Deeks et al., 2010; Dunaif, 1997; Elsenbruch et al., 2006; Ferriman & Gallwey, 1961; Kitzinger & Willmott, 2002; Mechanick & Dunaif, 1990; Moran et al., 2010; Polson et al., 1988; Sonino et al., 1993), other studies have not found a significant association between the physical symptoms of PCOS and symptoms of mood or anxiety (Barth et al., 1993; Hollinrake et al., 2007; Karjula et al., 2017; Shulman et al., 1992). In the current study, the finding related to significant improvement
in mood within the PCOS group appears to be associated with direct effects of anti-androgen treatment on the brain, since physical symptoms of PCOS including hirsutism, weight gain and acne may not necessarily change with treatment at the twelve-week mark.

There is very little available data from interventional studies related to the effect of treating testosterone-excess on symptoms of depression. It is also important to note that most studies until now have examined this relationship by including experimental study designs involving testosterone administration, to assess the effect of testosterone on mood and cognitive variables (see Chapter Five for a review). Prior research has shown that 0.5 mg of testosterone in reproductive-aged females results in an approximate ten-fold increase in blood Total Testosterone levels fifteen minutes post-administration, and a return to baseline levels in 90 minutes has been found (Bos et al., 2013; Hermans et al., 2007; Postma et al., 2000; Schutter & van Honk, 2004; Tuiten et al., 2000; van Honk et al., 2005; van Honk et al., 2001). However, this result is limited to Total Testosterone levels and the effect of testosterone administration in terms of Free Testosterone levels is still unclear. It is not reasonable to suggest that testosterone administration studies are likely to have the opposite results of anti-androgen administration (see Chapter Five). The former increases testosterone for only a short period of time. It is clearly not just the opposite of treatment for PCOS. However, these studies can provide useful data regarding the effects of androgens in the brain.

Only one small study so far has examined the effect of anti-androgen treatment on cognitive function and mood in females with PCOS (Schattmann & Sherwin, 2007a). Similar to the current study, Schattmann & Sherwin (2007a) examined the effect of anti-androgen treatment over a span of three months on mood and cognitive function in a sample of eight hirsute females with PCOS compared with 11 females with PCOS who did not receive anti-androgen treatment. In this small study, no significant differences were found on measures of mood (POMS-Bipolar Form) between the two groups at the baseline or the follow-up assessment. However, this study
did not have a healthy comparison group without PCOS. Additionally, this study used a self-report scale (POMS-Bipolar Form) which is suitable for detecting rapid changes in mood at a very low level. It is not a clinical scale, compared with the current study which used standardised clinician-rated and self-reported depression-rating scales (QIDS and HADS) that are better able to detect sensitivity of symptoms of depression at a mild-moderate level of severity. An important methodological issue with Schattmann & Sherwin’s (2007a) study was a small sample size (treatment group = 8, no-treatment group = 11) in contrast to the current study including 42 females with PCOS and 50 controls.

In summary, although there was only an indication of a difference but no significant difference between the PCOS and control groups on measures of mood and anxiety at the follow-up assessment, there was a significant improvement in symptoms of mood and anxiety within the PCOS group, most likely independent of significant change in physical symptoms, implying that anti-androgen treatment may benefit mood in females with PCOS.

8.7.1.2 Associations between mood, anxiety and cognitive variables: group comparisons and correlation analyses

The following sections will discuss findings related to differences between the PCOS and the control groups on changes in mood, cognitive function and emotion processing over time; and findings related to change in mood variables and change in cognitive and emotion processing variables within the PCOS group following anti-androgen treatment.

8.7.1.2.1 Verbal learning and memory

No significant differences were found between the PCOS and control groups on change in measures of verbal learning and memory over time. However, significant improvements were seen within the PCOS group on all CVC variables, except for recognition accuracy.
Additionally, no significant correlations were found between change in CVC variables and change in mood and anxiety variables following treatment in the PCOS group.

There is little evidence of change or improvement in verbal learning and memory in patients with PCOS following anti-androgen treatment. Verbal memory is considered a female-superior domain (Halpern, 2000) and studies have hypothesised that high androgen levels would show reduced function in this domain, however, the superior female performance is likely to be related to the organisational effects of testosterone and not ambient testosterone levels. Only one interventional study has examined the effect of anti-androgen treatment on verbal learning and memory (RAVLT, Logical Memory Test, and Paired Associates Test) and found no significant change following treatment (Schattmann & Sherwin, 2007a). However, this study included two groups of females with PCOS, did not have a comparison group of healthy females, and was very small. Analysis consisted of comparisons between two groups with PCOS exposed either to the treatment ($n = 8$) or placebo condition ($n = 11$). In the current study, although an improvement in an aspect of verbal learning and memory together with an improvement in symptoms of depression and anxiety was observed within the PCOS group following treatment, correlational analysis showed no significant association between change in mood and change in CVC variables. Changes in mood are, therefore, unlikely to explain these results.

8.7.1.2.2 Visuospatial learning and memory

No significant differences were found between the PCOS group and control group on change in measures of visuospatial learning and memory (GMLT) from the baseline to follow-up assessment. However, significant improvements were observed within the PCOS group on most GMLT variables following treatment. No significant correlations were found between change in mood and change in GMLT variables following anti-androgen treatment in the PCOS group.
Regarding visuospatial learning and memory, few studies have examined the association between testosterone levels and visuospatial learning and memory (Gómez-Gil et al., 2009; Pintzka et al., 2016; Postma et al., 2000). Two interventional studies have shown a positive effect of testosterone, with testosterone administration related to significant improvement in some aspects of visuospatial learning and memory (Pintzka et al., 2016; Postma et al., 2000). However in Pintzka et al’s (2016) study, only one aspect of spatial learning was found to improve (improved representation of directions assessed by the Virtual Environment Learning and Navigating Task: Sense of Direction Test), and no differences in changes in navigation ability and strategies were observed between the testosterone \( (n = 21) \) and placebo \( (n = 21) \) groups. Similarly, in Postma et al’s (2000) study, testosterone administration did not have a significant effect on the first two conditions of the Spatial Memory Stimulus Task (Object-to-position and the Positional Reconstruction condition), but had a positive effect on the combined condition following testosterone administration in this study. Furthermore, one small cross-sectional study found better visual memory (Visual Paired Associates) in a testosterone-treated group \( (n = 9) \) compared with the no-treatment group \( (n = 10) \) in female-to-male transsexuals (Gómez-Gil et al., 2009). Taken together, these results suggest that testosterone administration improves some aspects of visuospatial learning and memory. However, as discussed in the previous part of the current section, studies involving testosterone administration would not necessarily imply that anti-androgen treatment (involving blocking or reduction of testosterone levels) would have an opposite effect on spatial learning and memory in the form of a negative or deleterious effect. Thus, the current results including no significant group differences on change in GMLT variables are expected, since at baseline, no significant differences were found between the two groups on GMLT variables.
8.7.1.2.3 Psychomotor speed

Significant differences were found between the PCOS and control groups on change in one measure of psychomotor speed, the TCT. Following anti-androgen treatment, the PCOS group significantly improved on performance on the TCT compared with the control group. Additionally, the control group showed worse performance over time on the TCT (number of correct moves), which may be a chance finding, and which would have contributed to significant differences between groups. At baseline, the PCOS group showed significantly worse performance on the total number of correct moves on the TCT compared with the control group (ES = 0.70). However, no significant difference was observed within the PCOS group on change in performance on the TCT following treatment. Results, therefore, suggest that although significant differences were found between groups from baseline to follow-up assessment, this change was likely influenced by a worsening of performance observed in the control group over time. This result could be explained by the fact that the PCOS group may have been more motivated to perform well at the second testing session compared with the control group, considering that the former group received treatment (which may have been a motivating factor) in between the two sessions while the control group did not. Such a lack of motivation and investment in the control group, more likely to be experienced by the PCOS group, could be the reason for worse performance shown by the control group over time. Additionally, the TCT is a tedious and monotonous task requiring participants to chase a coloured moving tile, one tile at a time, through a 10 by 10 grid of grey tiles. Taken together, these reasons may help explain poorer performance on the TCT by the control group over time.

No significant differences were found between the two groups or within the PCOS group on change in the TMT - Part A performance from baseline to follow-up. Overall, current results do not suggest that anti-androgen treatment may change psychomotor function.

Significant correlations were found between improvement in symptoms of mood and anxiety and improved performance (more correct moves) on the TCT over time. A significant
correlation was also found between improved symptoms of depression (QIDS) and improved performance on the TMT - Part A. Thus, in the current study, improvements in mood and anxiety were associated with better performance on the two psychomotor speed task (TCT and TMT – Part A). These results are in line with a meta-analysis demonstrating significant correlations between depression severity and cognitive performance particularly in the domains of psychomotor speed, memory and executive function (McDermott & Ebmeier, 2009).

In the current study, at baseline, Free Testosterone and FAI levels significantly negatively correlated with performance on the TCT, indicating that higher testosterone levels were associated with worse performance on the TCT. It was, therefore, expected that treatment aiming to normalise testosterone levels would be followed by improvement in measures of psychomotor speed. However, contrary to this hypothesis, no significant difference was observed within the PCOS group on change in performance on the TCT following treatment. Two interventional studies examined change in psychomotor speed following anti-androgen treatment in females with PCOS ($n = 8$) (Schattmann & Sherwin, 2007a), and androgen treatment in female-to-male transsexuals ($n = 25$) (Slabbekoorn et al., 1999), and found no significant change in performance over time. In their small interventional study, Schattman and Sherwin (2007a) found no significant change in psychomotor speed (Manual Dexterity and Perceptual Speed :Finding A’s, Purdue Pegboard) in females with PCOS receiving anti-androgen treatment over a span of three months compared with an untreated group of PCOS females ($n = 11$) somewhat similar to the current study. In Slabbekoorn et al’s (1999) study, no significant effects were found on psychomotor speed (manual dexterity and perceptual speed: Fine motor Movement Task, Test D2 Task) following anti-androgen treatment in female-to-male transsexuals (Slabbekoorn et al., 1999). Consistent with these findings, the current study did not find any significant change within the PCOS group on measures of psychomotor speed following anti-androgen treatment. However, correlational analysis indicated that reduced
symptoms of depression and anxiety were significantly associated with aspects of psychomotor speed in the PCOS group.

8.7.1.2.4 Attention and executive function

No significant differences were found between the two groups, or within the PCOS group, on change in any attention and executive function variables between baseline and follow-up. This was expected since no significant differences were found between the two groups at baseline on attention and executive function variables. However, a significant correlation was found between improvement on symptoms of depression (QIDS) and improvement on the TMT- Part B (assessing visual attention and cognitive flexibility). Additionally, significant correlations were found between improvement in two Digit Span variables (Total Forward and Forward Span; assessing working memory) and improvement in depression (HADS Total) and anxiety (HADS-A) symptoms.

Interventional studies examining the effect of testosterone on attention and executive function suggest that testosterone may have a negative effect on verbal fluency (executive functioning) (Schattmann & Sherwin, 2007a). Two studies involving testosterone treatment found a worsening in performance in measures of verbal fluency (Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995), and one study involving anti-androgen treatment found improvement in performance on verbal fluency following treatment (Schattmann & Sherwin, 2007a). In a sample of hirsute females (Schattmann & Sherwin, 2007a), verbal fluency (COWAT) significantly improved following anti-androgen treatment ($n = 8$) compared with the placebo condition ($n = 11$), which may have been a chance finding, since in this study post-treatment FAI levels were not significantly correlated with improved verbal fluency. Thus, the observed improvement in performance in verbal fluency may not have been directly related to reduced FAI levels, also found in the current study. Reduced verbal fluency (Word and Sentence Production) was found following testosterone treatment in two longitudinal studies.
involving female-to-male transsexual samples (Van Goozen, Cohen-Kettenis, et al., 1994; Van Goozen et al., 1995). However, another study found no significant change in verbal fluency (Word and Sentence Task) following testosterone treatment in a sample of 25 female-to-male transsexuals (Slabbekoorn et al., 1999). All of the above-mentioned studies are, however, very small and potentially underpowered to generate findings.

To summarise, although no significant group differences were found in change in attention and executive function variables in the current study, results suggest that improvement in mood in the PCOS group was associated with improvement in performance on measures assessing cognitive flexibility (TMT – Part B) and working memory (Digit Forwards variables).

8.7.1.3 Associations between mood, anxiety and emotion processing variables: group comparisons and correlation analyses following anti-androgen treatment

8.7.1.3.1 Recognition accuracy and reaction time: group comparisons and correlations

No significant differences were found between the PCOS and control groups on change in recognition accuracy on the FER Task over the study period. However, a significant improvement was seen within the PCOS group following anti-androgen treatment in accurately recognising facial expressions of disgust. Significant differences were found between the PCOS and control groups on reaction time to fearful facial expressions over time. The PCOS group responded faster to fearful facial expressions compared with the control group over the study period. Within the PCOS group faster reaction time to all facial expressions with the exception of happiness was observed over treatment. As noted previously, it is possible that anti-androgen treatment results in increased emotional sensitivity by reducing or blocking testosterone levels, which may explain the current results including significant improvement in recognition accuracy related to disgusted facial expressions and reaction time related to fearful faces in the PCOS group. It could also be that significant improvement in symptoms of depression and
anxiety found in the PCOS group may be associated with improvement in aspects of emotion processing. In line with this hypothesis, significant correlations were found between improvement in symptoms of depression and anxiety (HADS) and improved recognition accuracy to facial expressions of negative faces (disgust and fear) in the PCOS group. However, no significant correlations were observed between change in mood and anxiety variables and change in reaction time on the FER Task in the PCOS group.

Emotion processing is a crucial function required for successful interpersonal relationships and includes the ability to detect and decode facial emotional expressions (M. Phillips et al., 2003). There appear to be emotion-specific deficits in MDD (Bourke et al., 2010), including impaired general recognition accuracy of sad and happy faces, misinterpretation of neutral faces as sad, and negative attentional biases to sad faces (Bouhuys et al., 1999; Bourke et al., 2010; Roiser & Sahakian, 2013; Stuhrmann et al., 2011). Douglas and Porter (2010) found a specific deficit in individuals with severe depression in the recognition of facial expressions of disgust compared with controls. This is consistent with the current findings of improvement in disgust recognition in the PCOS group following anti-androgen treatment, which was related to improved mood symptoms within the PCOS group over treatment. Disgust recognition has also been found to be impaired in unmedicated patients with Parkinson’s disease, indicating an association with dopamine dysfunction, which has also been implicated in severe depression (Dunlop & Nemeroff, 2007; Sprengelmeyer et al., 2003). However, the PCOS sample included in the current study did not have severe depression. One animal study found significantly lower levels of dopamine in testosterone-induced PCOS rats compared with controls, suggesting that a decrease in dopamine levels may be related to increased serum testosterone levels found in PCOS, and also related to the high occurrence of mood and anxiety disorders found in this population (Chaudhari & Nampoothiri, 2017). However, this needs further examination as the finding related to recognition accuracy of disgusted faces in relation to mood has not been commonly reported in other studies with milder depression (Bourke et al., 2010).
An advancement of behavioural paradigms related to emotion processing, together with testosterone administration studies, has made it possible to further examine the relationship between emotion processing and testosterone in some detail. Literature suggests that testosterone administration is associated with a reduced response to threatening or aversive facial stimuli (particularly facial expressions of fear and anger) a decrease in gaze avoidance and gaze aversion (reduced gaze or attendance away from threatening or negative faces, resulting into a more enduring attendance) toward angry faces following testosterone administration in healthy females; indicating a reduced anxiety response (Bos et al., 2016; Bos et al., 2013; Enter et al., 2015; Hermans et al., 2007; Moukheiber et al., 2010; Olsson et al., 2016; Terburg et al., 2012; van Honk et al., 2005; van Honk & Schutter, 2007; van Honk et al., 2011; van Wingen et al., 2009) (as discussed in detail in Chapter Five). However, findings directly relating testosterone administration to anxiety rating scales are not clear. Additionally, experimental studies involving supraphysiological doses of testosterone in healthy females only bring about a short-term elevation of testosterone levels compared with the more chronically elevated levels as seen in PCOS, as discussed in previous sections, and therefore, do not replicate the stable and high levels of Free Testosterone (hyperandrogenism) observed in PCOS which manifest as physical symptoms such as hirsutism, acne and alopecia. Moreover, some studies have suggested a curvilinear relationship between testosterone and cognitive function (Moffat & Hampson, 1996; Romero-Martínez et al., 2015) and mood (Weiner et al., 2004). This suggests that increases in testosterone levels are associated with better mood or cognitive performance up to a certain point, and subsequently cognitive performance or mood decreases with further increasing levels of testosterone. Therefore, it could be that the exogenous doses of testosterone in healthy females may reach the cut-off point to have the strongest effect on mood or cognitive function following which such an effect may not be observed. Thus, in females with PCOS, with chronically abnormally elevated testosterone levels compared with the short-term elevated levels observed in healthy females following supraphysiological doses
of testosterone, the relationship between cognitive function and testosterone levels may be more challenging to investigate clearly.

Regarding neuroimaging studies, only one study until now has examined emotion processing in females with PCOS (Marsh et al., 2013). In this small interventional study, baseline fMRI results showed greater limbic activation including areas implicated in depression such as the prefrontal cortex, anterior cingulate, amygdala, and ventral basal ganglia/nucleus accumbens (involved in the integration and evaluation of emotional information) in seven females with PCOS during an emotion processing task, compared with five healthy controls. However, activation reduced following Metformin treatment, which has been shown to be associated with decreased testosterone levels (Campagnoli et al., 2012; Kriplani & Agarwal, 2004). However, in Marsh et al’s (2013) study, post-treatment testosterone levels were not reported. Additionally, their study reported no differences between groups in performance on tasks assessing emotion processing involving pictures with emotional content.

A substantial number of studies have shown the modulatory actions of testosterone on both subcortical and cortical brain regions (van Wingen et al., 2011), and its association with increased amygdala activity during recognition of facial stimuli including angry and fearful faces (Bos, Hermans, Ramsey, & van Honk, 2012; Hermans et al., 2008; van Wingen et al., 2010) (see Chapter Five). Three brain-imaging studies involving testosterone administration in healthy females of reproductive-age found increased amygdala activity during emotion processing tasks, however, no significant change in performance on these tasks (emotion condition of the Blocked Design Task, Dynamic Facial Expression Task, adaptation of the RMET) was found (Bos et al., 2016; Bos et al., 2013; van Wingen et al., 2009). These results suggest that exogenous testosterone administration in healthy females with lower levels of testosterone, compared with females with PCOS with naturally elevated levels of testosterone, may affect amygdala activity differently; and that an increased activity may not lead to
substantial change in emotion processing measures. Conversely, anti-androgen treatment may decrease amygdala activity and, therefore, improve symptoms of low mood and improve performance on aspects of emotion processing, which was found in the current study.

Previous studies in depression have found more sensitivity and faster reaction time related to recognition of fearful faces in individuals with depression and relatives of individuals with depression (Le Masurier, Cowen, & Harmer, 2007). Findings include reduced fear recognition following antidepressant treatment, suggesting that increased recognition of fearful faces may be a trait marker for MDD which may be normalised following treatment (Bhagwagar et al., 2004), reviewed by Bourke et al. (2010). However, some studies have suggested that antidepressant treatment increases testosterone levels previously found to be lower in depressed samples (Cohen, 1999; Giltay et al., 2012; Kumsar et al., 2014), which may be responsible for changes in emotion processing. It has also been suggested that social intelligence or empathy is negatively associated with testosterone administration (Olsson et al., 2016; van Honk et al., 2011). The question remains whether the increase in testosterone levels following antidepressant treatment is related to the emotional numbing effect in some patients (Price, Cole, & Goodwin, 2009; Read, Cartwright, & Gibson, 2014). However, in the current study, testosterone levels were decreased/blockaded and not increased and, therefore, results suggest that improved recognition of threatening or aversive faces (fearful and disgusted) may be due to improvement in mood and anxiety, and not directly due to the change in testosterone levels.

To summarise, in the current study, significant differences were found between the two groups on change in reaction time related to fearful faces, with the PCOS group showing greater improvement in reaction time compared with the control group over the study period. No significant differences were observed between the two groups on recognition accuracy. Additionally, significant improvements were observed within the PCOS group in recognition accuracy related to disgusted faces and overall reaction time following anti-androgen treatment.
Significant positive correlations were found between improvements in symptoms of mood and anxiety (HADS) and improvement in recognition accuracy of fearful and disgusted faces on the FER Task in the PCOS group following anti-androgen treatment. These results suggest that an improvement in symptoms of depression and anxiety was associated with improved recognition of threatening or aversive facial expressions depicting fear and disgust.

8.7.1.3.2 Neutral misinterpretation bias: Group comparisons and correlations

In the current study, the PCOS group was less likely to interpret neutral faces as angry (weaker neutral misinterpretation bias), compared with the control group, over time. Additionally, a significantly weaker neutral misinterpretation bias related to angry faces was seen within the PCOS group following treatment. At baseline, no significant differences were found between the two groups in misinterpretation of neutral faces to anger, and therefore, this result is somewhat surprising. No significant correlations were observed between change in neutral misinterpretation bias and change in symptoms of depression or anxiety in the PCOS group.

The current result of a reduced tendency to misinterpret neutral faces as angry over time in the PCOS group could be related to lowered testosterone or blockade of testosterone receptors following treatment. This is, however, a tentative explanation, since no other studies have examined emotion processing in relation to testosterone levels in females with or without PCOS until now. Prior research has shown a relationship between testosterone and anger, with findings including a single administration of testosterone inducing cardiac accelerative responses to angry faces in healthy females (van Honk et al., 2001), reduced sensitivity to recognising angry faces (van Honk & Schutter, 2007), a general decrease in aversion from threatening stimuli including angry faces (Wirth & Schultheiss, 2007) and androgen-administration followed by an increase in anger–proneness in females (Van Goozen, Frijda, & Van de Poll, 1994). It could be that normalising testosterone levels in the current study decreased the hypervigilance or anxiety response, which is generally responsible for scanning and responding to neutral stimuli as
threatening. Such a decreased hypervigilant anxiety response may help explain the finding of a reduced or weakened neutral misinterpretation bias, of being less likely to interpret neutral faces as angry following treatment. In the current study, although a significant improvement was found in symptoms of depression in the PCOS group following treatment, correlational analysis did not show a significant association between change in performance on the neutral misinterpretation bias aspect of the FER Task and change in mood symptoms. Improvement in mood would reduce a negative response bias towards stimuli, however, no study so far has found a neutral misinterpretation bias toward angry facial expressions in individuals with depression (Bourke et al., 2010). Consistent with these results, the current study did not find a significant correlation between change in symptoms of mood and change in neutral misinterpretation bias in the PCOS group over time.

8.7.1.3.3 Reading the Mind in the Eyes Test

No significant differences were found between the PCOS and control groups, or within the PCOS group, on change in RMET performance over time. No significant correlations were observed between the change in RMET score and the change in mood and anxiety variables in the PCOS group following treatment.

The RMET has not been used in a PCOS sample until now. It has previously been used by two interventional studies which found worse performance (lower empathy) on the RMET following testosterone administration, compared with the placebo condition, in healthy reproductive-aged females (Olsson et al., 2016; van Honk et al., 2011). Van Honk et al. (2011) in a double blind placebo controlled within subjects cross-over study including a group of 16 females in total, reported a significant decrease on the RMET total score following testosterone administration compared with the placebo condition suggesting that testosterone administration was associated with worsened emotion processing. In the same study, a significant negative correlation was found between endogenous (baseline) Free Testosterone levels and RMET
scores in the placebo condition, suggesting an organisational effect of testosterone, in that higher levels of Free Testosterone were associated with worse scores on the RMET (lower empathy). Another randomised double-blind study including a total of 33 female participants exposed to both testosterone and placebo conditions found similar results, with significantly worse performance (less accuracy) on the RMET following testosterone administration compared with placebo condition (Olsson et al., 2016). Of interest, no significant effect was found on self-reported anxiety and/or mood following testosterone administration in these two studies (STAI, POMS). Results, thus, indicate that increasing testosterone levels in females may be related to worsening of “social intelligence” or empathy. Testosterone has also been shown to decrease females’ empathic mimicry of emotional facial expressions (Hermans et al., 2006). In the current study, significant differences were found between the two groups on performance on the RMET at baseline, with the PCOS group performing significantly worse compared with the control group. It would, therefore, be hypothesised that performance on the RMET would improve following anti-androgen treatment. However, contrary to this expectation, no significant differences between the two groups were found over time. Additionally, improvement in mood was not found to be significantly correlated with change in performance on the RMET, suggesting no significant association between reduced symptoms of depression and improvement in empathy or social intelligence. It could be that the RMET is not sensitive enough to detect changes in relation to treatment response, which may explain current results with non-significant results.

8.8 STRENGTHS AND LIMITATIONS OF THE CURRENT STUDY

The current study investigated mood, anxiety, cognitive function and emotion processing in females with PCOS following anti-androgen treatment, compared with a non-PCOS comparison group which did not receive such treatment, over twelve weeks. The longitudinal
study design is a significant strength of the current study as it helped to analyse change in symptoms of mood, anxiety cognitive function and emotion processing, over time, for the patient group, and the entire sample as a whole, which would not have been possible in a cross-sectional study. Additionally, the current study is also the first one to assess the relationship between change in mood and anxiety and change in cognitive function and emotion processing following anti-androgen treatment in the PCOS sample. However, limitations of the current study will be discussed in the following section.

8.8.1 Limitations

8.8.1.1 Anti-androgen medication

After the first consultation with the Gynaecological-Endocrinologist, patients were prescribed medication including CPA, Spironolactone, OCPs and/or Metformin depending on their symptom profile. Medication was altered over the 12 week period as considered clinically appropriate. Thus, the PCOS sample differed substantially in the type and dose of the hormonal medication taken. Heterogeneity in hormonal medication meant that mood and cognitive tests which may have been sensitive to a certain type of anti-androgen agent were unlikely to have been identified within the whole PCOS sample. However, the current study did not aim to examine the effects of specific anti-androgen agents on mood and cognitive function, rather it focused on broad treatment response. It would have been unethical to treat all patients with the same anti-androgen agent, and findings from such a study would not have been generalisable to other PCOS samples.

8.8.1.2 Androgen levels at follow-up

One of the limitations of the current study was that androgen measures were not collected at the time of the follow-up assessment. The reason for this was that the treatment involved in the study included multiple anti-androgen agents (see Chapter Three and Chapter Eight, Section
8.2.2). Such anti-androgen agents have different mechanisms and correct symptoms of PCOS by either reducing or blocking testosterone levels. Therefore, post-treatment androgen levels may not necessarily have been decreased if the anti-androgen agent aimed to block levels rather than decrease testosterone levels.

8.8.1.3 Study investigator

The primary investigator in the current study (the author) was not blind to whether participants were in the PCOS or the control groups. The investigator was responsible for almost all aspects of the current study. This raised the possibility for an investigator bias to occur during the testing. For most cognitive measures, investigator bias was not possible as data were recorded automatically on computer software. The measure with greatest potential for bias by the investigator was the QIDS, a clinician-rated scale for assessing symptoms of depression. For the first assessment, since the investigator was aware of the group the participant belonged to, this could have potentially contributed to QIDS ratings. However, the current study also included the HADS, which is a self-report measure. This would reduce the possibility for bias in the overall assessment of symptoms of depression.

8.8.1.4 Menstrual cycle

Studies have shown that testosterone levels in females are subject to small but significant fluctuations during different menstrual phases (Goebelmann et al., 1974; Judd & Yen, 1973) (See Chapter 5, Section 5.3). Goebelmann et al. (1974) found Total Testosterone levels were highest around midcycle LH peak, and higher during the follicular compared with the luteal phase of the cycle. However, Free Testosterone levels were not assessed in this study. A recent review and meta-analysis did not find robust evidence related to the influence of menstrual cycle on cognitive function (Poromaa & Gingnell, 2014).
In the current study, it was impossible to match PCOS participants and non-PCOS control participants for phase of menstrual cycle since females with PCOS experience oligomenorrhea (absent or irregular menstrual period) which is also one of the diagnostic features of the syndrome (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Data related to menstrual phases was collected for all control participants at both time points, and results showed that majority of the participants were in the luteal phase at both cognitive assessments (See Section 8.2), which would have controlled for any differences that may contribute to cognitive outcome variables.

8.8.1.5 Sample Size/Power

The current study (including 42 patients and 50 controls) had 80% power to show an effect size difference between groups of 0.6 (or above) as significant (two-tailed $\alpha=0.05$). Therefore, if the obtained effect sizes in the current results were smaller than 0.6, the study was not adequately powered to show a statistically significant difference. A larger sample size would be recommended for future research, which will be discussed in Chapter Nine (see Section 9.5.1). It is important in the context of the current study to consider the possibility that the testing of multiple outcome variables may lead to the possibility of Type 1 errors, which means that statistically significant results could be chance findings. While acknowledging that many outcomes have been tested in this PCOS study, many of the outcomes are tested to confirm previous study results and additionally, individual statistically significant results are not discussed unless they fit into a coherent pattern with other significant results. The exploratory nature of much of the testing of associations is also acknowledged and significant results will need to be confirmed in future studies.
8.9 CONCLUSION

Anti-androgen treatment over a period of twelve weeks was administered to females with PCOS. Mood and cognitive assessment was carried out before and following treatment. Symptoms of depression were found to improve following anti-androgen treatment within the PCOS group, however, no significant differences were found on change in mood between groups over the study period. No significant improvements were observed in symptoms of anxiety following anti-androgen treatment in the PCOS group compared with the control group. Significant differences were found between the two groups on a measure of psychomotor speed (TCT), with the PCOS group showing an improved performance over time. Additionally, reaction time related to recognising fearful faces on the FER Task improved in the PCOS group compared with the control group, and a neutral misinterpretation bias toward angry faces was found to improve (weaker bias) in the PCOS group compared with the control group over time. The PCOS group showed significant improvements in cognitive domains including verbal learning and memory, visuospatial learning and memory, and recognition accuracy of disgusted facial expressions, and overall reaction time (with the exception of happy faces) on the FER Task. It is possible that such an improvement in aspects of cognitive function may be associated with the effect of anti-androgen treatment in reducing the androgenic action on the brain, by reducing or blocking testosterone levels. However, it is also likely that improvement in aspects of cognitive function and emotion processing may be associated with improvement in symptoms of depression and anxiety, also observed following anti-androgen treatment in the patient group. The two effects are, however, difficult to disentangle. An improvement in tasks assessing specific aspects of cognitive function and emotion processing may be related to improvements in symptoms of mood and anxiety, or normalisation of androgen levels, or both of these factors.

Post-treatment correlational analyses showed significant correlations between improvements in symptoms of depression and anxiety and improvements in performance on psychomotor speed
variables, and attention and executive function variables (specifically, working memory and cognitive flexibility). Correlational analysis also suggested significant associations between improvement in symptoms of depression and anxiety and improvement in recognition accuracy of fearful and disgusted facial expressions. This is the first study to examine the association between change in mood and change in cognitive function and emotion processing following anti-androgen treatment in females with PCOS. The following chapter will include an overall summary of findings from both baseline and longitudinal findings of the current study, and will discuss implication for future research.
CHAPTER 9

SUMMARY AND CONCLUSIONS

9.1 STUDY OVERVIEW

This thesis has presented findings from an observational, longitudinal study examining mood, anxiety, cognitive function and emotion processing in relation to testosterone levels in females of reproductive age with and without PCOS. The overall aim of the current study was to determine whether there was a direct relationship between androgen levels and mood, anxiety, cognitive function and emotion processing in females with PCOS, with abnormally elevated testosterone levels. In order to achieve this aim, the current study assessed androgen levels, symptoms of depression and anxiety, and associated cognitive function and emotion processing performance in females newly referred to a gynaecological-endocrine clinic in Christchurch, New Zealand. At baseline, correlations between androgen levels and symptoms of depression and anxiety and cognitive function and emotion processing were assessed across the whole sample. Inclusion of the entire sample with PCOS and non-PCOS control groups as a whole was beneficial, as this provided an opportunity to investigate the relationship between testosterone and mood and cognitive variables with the use of a broad range of androgen levels. Following this, an examination of the effects of standard clinical treatment of PCOS on symptoms of depression, anxiety, cognitive function and emotion processing was carried out by testing the group of females with PCOS before and following 12 weeks of anti-androgen treatment.

For some females with depression, there may be an underlying hormonal abnormality, in the form of androgen excess. Therefore, it was anticipated that the results from the current research
may help benefit not only females with PCOS to select the most suitable treatment options, but also females with depression and abnormal androgen levels, who present to mental health services to be treated appropriately.

Another aim of the study was to facilitate collaboration between psychiatrists and endocrinologists. Females with PCOS who are referred to endocrinologists often report difficulty with mood and anxiety. Results from this study indicate that normalising androgen levels improves symptoms of depression in females with PCOS. Results also suggest that cognitive function and emotion processing, which are associated with symptoms of depression, may be benefited, to a certain degree, from anti-androgen treatment in females with abnormal androgen levels.

The main findings from the current study will be discussed in the following section.

9.2 PRINCIPAL FINDINGS

9.2.1 Baseline findings

As expected, androgen levels were significantly higher in females with PCOS compared with controls. Verbal IQ (assessed by NART) was significantly different between the two groups, due to the nature of the control group. Females with PCOS had a lower estimated verbal IQ compared with control females, therefore, NART was considered as a covariate in final analyses using ANCOVA.

9.2.1.1 Correlational findings

After controlling for NART, the following significant correlations were found:

- Higher FAI levels were significantly correlated with greater symptoms of depression (QIDS and HADS).
Higher Free Testosterone and FAI levels were significantly correlated with worse verbal learning and memory (worse performance on recognition accuracy on the CVC Task).

Higher Free Testosterone levels were significantly correlated with worse visuospatial learning and memory (worse performance on GMLT variables [total errors on GMLT Trial 5 and GMLT Total Learning]).

Higher Free Testosterone and FAI levels were significantly correlated with worse psychomotor speed (worse performance on the TCT [number of correct moves]).

Higher FAI levels were significantly correlated with worse aspects of facial emotion processing (worse performance in recognising fearful facial expressions on the FER Task).

The current study provides evidence for a significant association between testosterone levels, specifically, FAI levels, and mood, in that higher FAI levels were associated with higher symptoms of depression across the entire sample (n = 103). Additionally, higher levels of Free Testosterone and/or FAI levels were associated with worse performance on cognitive domains including verbal learning and memory (CVC recognition accuracy), visuospatial learning and memory (GMLT errors), psychomotor speed (number of correct moves on the TCT) and emotion processing (recognition accuracy of fearful faces). However, correlations were not consistent across all measures in these domains of functioning.

9.2.1.2 Multivariate regression analysis findings

Multivariate regression analysis was conducted to further determine whether the association between testosterone variables (Free Testosterone and FAI levels) and cognitive variables was independent of other relevant factors, including BMI, NART, age and symptoms of depression (HADS-D score). Free Testosterone levels were significantly associated with psychomotor
speed (TCT) \( p = 0.03 \) when BMI was excluded from the analysis. Other correlations in multivariate regression analysis were not found to be significant.

9.2.1.3 Correlations between mood and cognitive variables

Additionally, the analysis examining the association between mood and cognitive variables showed:

- A significant correlation between worse verbal learning and memory (CVC Trial 1) and greater symptoms of depression (HADS-D).
- A significant correlation between worse visuospatial learning and memory (GMLT Trial 5) and greater symptoms of anxiety (HADS-A).
- Significant correlations between worse performance on attention and executive functioning variables (Digit Span Test variables assessing working memory) and greater symptoms of depression (HADS, HADS-D).

These results suggest that higher levels of depression and anxiety are associated with worse selective aspects of cognitive function, particularly verbal and visuospatial learning and memory and attention and executive function.

9.2.1.4 Comparative findings

Significantly greater symptoms of depression were found in the PCOS group compared with the control group. No significant differences were found between groups on symptoms of anxiety. Significant differences were found between the PCOS and control groups on some cognitive measures, including worse performance by the PCOS group on psychomotor speed (TCT), worse (lower) recognition accuracy on fearful faces and worse RMET (social intelligence) total score after controlling for NART.

Overall, the hypothesis that females with PCOS will show significant more symptoms of depression compared with control participants was supported by the current study.
Additionally, the hypothesis that higher androgen levels will be significantly associated with higher symptoms of depression was partially supported. The hypothesis that androgen levels will be significantly negatively correlated with worse performance on tasks assessing cognitive function and emotion processing was partially supported.

9.2.2 Follow-up findings

9.2.2.1 Correlational analyses

Correlational analyses showed:

- Greater improvement in symptoms of depression and anxiety significantly correlated with improvement in performance on the TCT (more number of correct moves), assessing psychomotor speed.
- Improvement in mood symptoms (QIDS) significantly correlated with improved performance on the TMT - Part A (time), assessing psychomotor speed.
- Improvement in mood symptoms (QIDS) significantly correlated with improved performance on the TMT- Part B, assessing attention and executive function (specifically, cognitive flexibility).
- Improved symptoms of depression and anxiety (HADS) significantly correlated with improved performance on Digit Span Forwards variables (Total Forward and Forward span), assessing attention and executive function (specifically, working memory).
- Improvements in symptoms of depression and anxiety (HADS) significantly correlated with improved recognition accuracy related to fearful faces on the FER Task.
- Improvements in symptoms of depression (HADS-D) significantly correlated with improved recognition accuracy of disgusted faces on the FER Task.
9.2.2.2. Comparative analysis

Symptoms of depression were found to improve in the PCOS group compared with the control group over time, at trend level \((p < 0.06)\). No significant improvements were observed in symptoms of anxiety following anti-androgen treatment in the PCOS group compared with the control group. Within the PCOS group, a significant improvement was found in symptoms of depression and anxiety following anti-androgen treatment.

Over the study period, significant improvements were observed in some cognitive measures in the PCOS group compared with the control group. These measures included the TCT (number of correct moves), reaction time to fearful faces in the FER Task, and neutral misinterpretation bias related to angry faces (i.e., the PCOS group being less likely to interpret neutral faces as angry over time, compared with the control group). Within the PCOS group, significant improvements were observed following anti-androgen treatment in cognitive domains including verbal learning and memory (CVC Task) and visuospatial learning and memory (GMLT). Significant improvements were also observed on recognition accuracy related to disgusted faces, overall reaction time on the FER Task, and a reduced tendency to interpret neutral faces as angry (weaker neutral misinterpretation bias), compared with baseline performance in the same group.

Interpretation and discussion of specific study findings were presented at the end of the two previous chapters. The next section presents an overview of the current findings in relation to previous studies (see Table 9.1 and Table 9.2), followed by overall implications of the current research.
Table 9.1

*Comparison of Current Findings Related to Mood and Anxiety in Females with Polycystic Ovarian Syndrome with Previous Studies*

<table>
<thead>
<tr>
<th></th>
<th>Cross-sectional studies</th>
<th>Interventional studies</th>
<th>Current study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mood</strong></td>
<td>In PCOS samples, three studies found a positive association between FAI and symptoms of depression (n = 580), one found a negative association (n = 35), one found a curvilinear association. Seven studies including PCOS samples found no significant association (n = 541).</td>
<td>-</td>
<td>FAI levels significantly positively correlated with symptoms of depression (QIDS, HADS) indicating higher testosterone levels were associated with higher symptoms of depression.</td>
</tr>
<tr>
<td><strong>Anxiety</strong></td>
<td>In PCOS samples, five studies (n = 778) found a significant positive association between state anxiety and FAI levels, one found a negative association (n = 27), six remaining studies including PCOS samples found no significant association (n = 409).</td>
<td>-</td>
<td>No association between Free Testosterone or FAI levels and symptoms of anxiety at baseline.</td>
</tr>
</tbody>
</table>

307
Table 9.2  
Comparison of Current Findings Related to Cognitive Function and Emotion Processing with Previous Studies including Females With and Without Polycystic Ovarian Syndrome

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Cross-sectional studies</th>
<th>Interventional studies</th>
<th>Current study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal learning and memory</td>
<td>Two of seven studies found a significant relationship ( n = 49 ), one a negative relationship ( n = 25 ) (Wechsler Memory Scale), while the other a curvilinear association between Free Testosterone and verbal memory ( n = 24 ) (RAVLT).</td>
<td>One study assessed verbal learning and memory (RAVLT, Logical Memory Test, Paired Associates Test) and found no significant changes following anti-androgen treatment in a PCOS sample ( n = 8 ).</td>
<td>Significant negative correlation between Free Testosterone, FAI and CVC Recognition accuracy, suggesting that higher testosterone levels were associated with worse verbal learning and memory.</td>
</tr>
<tr>
<td>Visuospatial learning and memory</td>
<td>One of four studies found significantly better visual memory performance (Visual Paired Associates) at baseline in the testosterone-treated group ( n = 9 ) compared with the no-treatment ( n = 10 ) group.</td>
<td>One study ( n = 8 ) found better performance on one aspect of a visuospatial learning and memory task (combined condition assessing immediate and delayed recall) following testosterone administration in healthy females, another study found better spatial learning and memory (Virtual Environment Learning and Navigating Task) in the testosterone group ( n = 21 ) compared with controls ( n = 21 ).</td>
<td>Significant negative correlation between Free Testosterone levels and GMLT performance (total errors on GMLT Trial 5 and GMLT Trials 1-5), indicating higher testosterone levels were associated with worse spatial learning and memory.</td>
</tr>
<tr>
<td>Psychomotor speed</td>
<td>One study found a negative correlation between FAI levels and motor speed (Purdue Pegboard Task) in a PCOS sample ( n = 28 ), while another found a positive correlation between Free Testosterone and processing speed (Dart-throwing Task) ( n = 30 ).</td>
<td>No significant change in psychomotor speed following testosterone administration was found by two studies (one including a PCOS sample).</td>
<td>Significant negative correlations between Free Testosterone, FAI levels and performance on the TCT (number of correct moves). At follow-up, a significant positive correlation between improvement in psychomotor speed (TCT, TMT - Part A) and improvement in the symptoms of depression (QIDS).</td>
</tr>
</tbody>
</table>
Table 9.2 Continued

<table>
<thead>
<tr>
<th>Attention and executive function</th>
<th>No evidence of an association found by five studies ($n = 149$).</th>
<th>Results from two studies indicate worse performance on measures of verbal fluency following testosterone administration, however, one further study found no change in verbal fluency following testosterone treatment.</th>
<th>No significant associations were seen between attention and executive function variables and Free Testosterone/FAI levels at baseline. At follow-up, a significant positive correlation between improvement in attention and executive function (TMT - Part B) and improvement in the symptoms of depression (QIDS). Significant positive correlation between improvement in executive function (Digit Span variables) and improvement in symptoms of depression and anxiety (HADS).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotion processing</td>
<td>One study found a negative association between emotion processing (attending away from angry faces) and testosterone levels; however, only limited to testosterone samples collected six hours prior to testing.</td>
<td>Two studies found decreased empathy or “social intelligence” following testosterone administration in healthy females.</td>
<td>Significant negative correlation between FAI levels and recognition accuracy of fearful faces, indicating that higher testosterone levels were associated with worse performance in recognising fearful facial expressions on the FER Task. At follow-up, a significant positive correlation between change in recognition accuracy of fearful and disgusted faces and change in the symptoms of depression and anxiety (HADS), indicating that as symptoms of depression improved recognition accuracy also improved.</td>
</tr>
</tbody>
</table>
9.3 IMPLICATIONS OF THE CURRENT RESEARCH

The current project investigated the relationship between two very common and debilitating conditions in females, which while usually treated separately, were found to be closely linked. The purpose of this research was to examine, in detail, associations between androgens and symptoms of depression and anxiety and associated cognitive function and emotion processing in females of reproductive age.

The current study was able to show significant correlations between higher testosterone levels (FAI) and lower mood. Additionally, high levels of FAI and/or Free Testosterone were significantly correlated with aspects of worse cognitive function including worse verbal learning and memory, visuospatial learning and memory, psychomotor speed and worse emotion processing (recognition accuracy of fearful and sad faces). Following anti-androgen treatment, symptoms of depression improved in the PCOS group and some aspects of cognitive function improved. While these are encouraging findings, there was less evidence that these cognitive domains improved significantly more in the PCOS group compared with the control group. Despite the symptom profile of patients with PCOS, including hirsutism, acne, alopecia, skin thickening, and obesity, not changing drastically over the period of twelve-weeks of treatment, improvements in symptoms of depression and cognitive function were observed. These results indicate that treating high testosterone levels could directly benefit mood and associated cognitive impairment, and change in mood may not be solely due to change in unwanted physical symptoms (which may not occur at the twelve-week mark).

Overall, results from the study related to anti-androgen treatment benefitting mood and associated cognitive impairment are encouraging enough to warrant further investigation. These results also indicate that routine evaluation of endocrine status is important in females with depression.
9.4 FUTURE RESEARCH

9.4.1 Sample size

It was the intention of the current study to include a larger sample, however, this was challenging since at the beginning of the study, the Canterbury District Health Board (CDHB) published new “pathways” for General Practitioners mandating that the first-line of treatment for patients with PCOS include OCPs/COCPs following which referrals would be considered, which affected the sample size included in the study (see Chapter Seven, section 7.15.1.7). Future research would be benefitted by including a larger sample which would provide greater power for conducting statistical comparisons for outcome measures.

9.4.2 Longer-term follow-up

It would be useful to study the longer-term effects of anti-androgen treatment on mood and cognitive function in females treated for PCOS. The only two studies that have assessed the effect of anti-androgen treatment on mood and cognitive function in females with PCOS have assessed these functions at baseline and following three months of treatment. However, since the physical symptoms involved in the syndrome may take longer to be treated and to show a visible difference, it would be of interest whether resolution of these symptoms has an impact on improvement in symptoms of depression. It is also possible that cognitive function may take longer to improve. Thus, in future studies, a further longer-term (six-month or a twelve-month) follow-up would be valuable to determine stability or change in symptoms of mood, anxiety and cognitive function.
9.4.3 Cognitive measures

Most cognitive measures included in the current study did not improve differentially in PCOS patients compared with control participants over the course of anti-androgen treatment for PCOS. However, a significant improvement was observed within the PCOS group on most cognitive and some emotion processing measures. A suggestion for future research would be to include tasks assessing visuospatial ability as part of the test battery, as prior research has shown a positive association between testosterone levels and spatial ability.

9.4.4 Neuroimaging techniques

Longitudinal neuroimaging studies could provide valuable information about the effect of anti-androgen treatment on the brain. Until now, only one small neuroimaging study (Marsh et al., 2013) has examined emotion processing in females with PCOS and found significant results including 1) greater limbic activation in insulin-resistant PCOS females compared with controls, which resolved after Metformin treatment and, 2) positive correlations between fMRI limbic activation during emotion processing and mu-opioid binding potential. Future research may be benefitted by incorporating neuroimaging data together with cognitive assessment in samples with PCOS.

9.4.5 Extending findings to other populations

It may be useful to examine the percentage of female outpatients with depression who have abnormal androgen levels and the percentage of patients with treatment-resistant depression who have PCOS or PCOS-like symptoms. Since the current study found significant correlations between androgen levels and symptoms of depression, it would be of interest and value to investigate androgen abnormalities in females with depression. Additionally, it would be of interest to examine androgen levels in patients with depression who are treated with SSRIs and who have
experienced emotional numbing. This may elucidate whether SSRIs do in fact increase levels of testosterone and whether this is a significant factor in causing emotional numbing in some patients.

9.5 CONCLUSION

The current thesis has presented findings from a longitudinal study assessing PCOS patients and control participants on a broad range of mood and anxiety scales, cognitive tests, and emotion processing measures. This is the first study to simultaneously examine changes in mood, cognitive function and emotion processing following anti-androgen treatment in females with PCOS. Data support the hypothesis that females with PCOS experience more symptoms of depression and show worse cognitive function compared with non-PCOS control females. Data also support the hypothesis that chronically elevated androgen levels, specifically, Free Testosterone and FAI levels, characteristic of PCOS, are associated with more symptoms of depression and associated worsening in cognitive function, and that normalising androgen levels benefits symptoms of depression and improves aspects of cognitive function and emotion processing in females with PCOS.
REFERENCES


doi:10.1210/edrv.21.4.0401

doi:https://doi.org/10.1016/S0015-0282(02)04914-2


323


Chen, Z., Zhao, H., He, L., Shi, Y., Qin, Y., Shi, Y., ... Zhao, Y. (2011). Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nature Genetics, 43*(1), 55-59. doi:10.1038/ng.732


doi:10.3109/01674829309084450


doi:10.1210/er.2011-1034


doi:10.1210/jcem.84.11.6148


doi:10.1016/j.molmed.2006.05.006


doi:10.1177/107319119800500407


doi:10.1186/2040-2392-4-33


doi:10.1159/000345567


doi:http://dx.doi.org/10.1016/0002-9378(81)90746-8


doi:http://dx.doi.org/10.1016/j.psyneuen.2007.08.006


doi:http://dx.doi.org/10.1016/S0022-3999(96)00216-4


Hong, Y., Sung, J., Hong, Y. S., Jeong, K., Chung, H., & Lee, H. (2017). Polycystic ovary morphology is associated with insulin resistance in women with polycystic ovary


Horton, R., & Tait, J. (1966). Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. *Journal of Clinical Investigation, 45*(3), 301.


relation to affective symptoms in women with polycystic ovary syndrome. *Psychoneuroendocrinology, 36*(10), 1470-1479. doi:10.1016/j.psyneuen.2011.04.001


Khomami, M. B., Tehrani, F. R., Hashemi, S., Farahmand, M., & Azizi, F. (2015). Of PCOS symptoms, hirsutism has the most significant impact on the quality of life of Iranian women. PLOS One, 10(4), e0123608.


Klojčnik, M., Kavcic, V., & Vukman, K. (2017). Relationship of depression with executive functions and visuospatial memory in elderly. The International Journal of Aging and
doi:10.1016/j.jacc.2015.12.005

doi:http://dx.doi.org/10.1016/j.yhbeh.2006.02.006


doi:10.1089/jwh.2012.3479


Mansson, M., Holte, J., Landin-Wilhelmsen, K., Dahlgren, E., Johansson, A., & Landen, M. (2008). Women with polycystic ovary syndrome are often depressed or anxious--a case
control study. *Psychoneuroendocrinology, 33*(8), 1132-1138.
doi:10.1016/j.psyneuen.2008.06.003


https://doi.org/10.1093/humrep/dep399


doi:http://dx.doi.org/10.1016/j.fertnstert.2013.02.054


doi:https://doi.org/10.1016/S0015-0282(16)45254-4


In *Philosophical Transactions of the Royal Society B*, 371.
https://doi.org/10.1098/rstb.2015.0106


doi:10.1016/j.biopsycho.2009.03.004


doi:10.1016/s0140-6736(08)60488-2

doi:http://dx.doi.org/10.1016/0278-5846(87)90048-0

doi:https://doi.org/10.1016/j.mppsy.2008.12.001


doi:10.1186/2045-5380-1-10


https://journals.sagepub.com/doi/10.2466/pms.2001.92.3.857


Trivedi, M., Rush, A., Ibrahim, H., Carmody, T., Biggs, M., Suppes, T., . . . Dennehy, E. (2004). The Inventory of Depressive Symptomatology, Clinician Rating (IDS-C) and Self-Report (IDS-SR), and the Quick Inventory of Depressive Symptomatology, Clinician Rating (QIDS-C) and Self-Report (QIDS-SR) in public sector patients with mood disorders: a psychometric evaluation. *Psychological Medicine, 34*(01), 73-82.


(PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. PLOS One, 6(11), e27286.


APPENDICES
APPENDIX A

Information Sheet for the Study
A study investigating the relationship between sex hormones, mood and cognitive functioning in females attending gynaecological endocrine clinics in Christchurch

Principal Investigator: Professor Richard Porter
(richard.porter@otago.ac.nz, 0272900960)

Information Sheet
University of Otago, Christchurch – Department of Psychological Medicine

Introduction

You are being invited to take part in a research study. It is important for you to understand why the research is being done and what it will involve. Please take the time to read over this information sheet carefully and to ask us if there is anything that is not clear to you or if you would like more information. You are free to discuss this study with others to help you come to a decision. You are welcome to ask a support person to join you in asking further questions about the study. This person may be a family member, a friend or a professional involved in your care.

What is the purpose of the study?

The current study will examine the link between sex hormone levels, mood and cognitive functioning in women. We believe that by understanding the relationship between sex hormones and mood symptoms, more suitable treatment options may be developed.

If you agree to participate in the present study, you will complete several tasks measuring memory, learning, verbal fluency and how you process emotions. You will also be clinically
assessed for mood symptoms during your medical appointment at the Gynaecological Endocrinology Clinic.

**Who is running the study?**

This research is being conducted by Professor Richard Porter, Dr. Katie Douglas, Dr. Anna Fenton and Ms. Mayouri Sukhapure of the Department of Psychological Medicine, University of Otago, Christchurch.

**Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide to take part you are free to withdraw at any time without giving a reason, and this withdrawal will not result in any disadvantage to yourself or your healthcare. The data collected from you will be immediately destructed in such an event.

**If I participate, what will I be asked to do?**

If you indicate willingness to take part, then the PhD Student will ask you some more questions to check that you are suitable for the study. If so, you would have an opportunity to ask questions and if satisfied, to sign a consent form.

We will ask you about your age and ethnic background, and will assess your mood and cognitive functioning, both at baseline and three months later. As a part of your routine clinical care, we will collect information about your general health and take some blood tests to assess hormone levels and your metabolism.

The cognitive tests will involve pen-and-paper tests and computerised tests assessing your verbal learning and memory, visual learning and memory, and your emotion processing ability. These assessments will be carried out by the PhD student. For example, you will be asked to listen to a list of words and will then be asked to recall them. During the course of the study, you will be assisted and given clear instructions regarding the procedures involved, which will aid your understanding of the tasks. The total duration of the assessment would require around 1.5 hours of your time.

The results obtained from the study will be treated confidentially. You will be allocated with a research code number, protecting your anonymity. The results may be used for further research purposes only with your consent.

**What about my usual treatment?**

You will keep taking your usual medication which will be monitored by one of the endocrinologists involved in the study.
What are the possible advantages of taking part?

The information we obtain from this study may help us to improve treatment in the future for patients with hormonal problems, which may be linked to mood and changes in brain function. At present, we have no information about whether the standard treatments for hormone imbalances help mood or brain function. The current study would help contribute to this knowledge.

What are the risks involved in taking part in the study?

We do not foresee any risks in participation. However, a clinician will be contacted if you became distressed during assessment or testing.

Will I receive compensation for the time taken to be part of this study?

You will receive compensation in the form of a $20 petrol voucher, to assist you in covering your travel related expenses.

Will my taking part in this study be kept confidential?

The current study is conducted within the Clinical Research Unit, in collaboration between the University of Otago, Christchurch and the Canterbury District Health Board. Two types of information will be collected from you – research information and clinical information. Research information is kept in a non-identifying form as described above. Usual CDHB policies are followed for confidentiality of clinical information. Health data is required to be stored for 10 years.

All material that you provide us will be treated in the utmost confidence. We will hold research information about you on a computer in the Department of Psychological Medicine in Christchurch. The study has a security system which ensures that all information you provide is stored in anonymous form on computer files and that no data that can be linked to an individual can be accessed without knowledge of this security system. Only those directly involved in the study will have access to this information and we will ensure that confidentiality is kept. Your identity will not be revealed in any reports based on this study.

If during the course of the study, we discover information which is important to your continued health and safety, we will discuss this with you and ask your permission to convey this to your endocrinologist.

What will happen to the results of the research?

We plan to finish the study by the end of 2018 and to submit the results for publication in scientific journals and to present results at international conferences. No-one will be identified in these reports.
Where can I get information about the study?

Mayourri Sukhapure can be contacted by telephone on 0273076502 or e-mail on sukma003@student.otago.ac.nz.

“This study has been approved of by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence and investigated, and you will be informed of the outcome”.
APPENDIX B

Consent Form for the Study
A study investigating the relationship between sex hormones, mood and cognitive functioning in females

Principal Investigator: Professor Richard Porter
(sukma003@student.otago.ac.nz, 0272900960)

CONSENT FORM FOR PARTICIPANTS
University of Otago – Department of Psychological Medicine

PARTICIPANTS NAME:

- I have been invited to take part in a study investigating whether sex hormones are associated with mood and cognitive functioning. This research is being conducted by Professor Richard Porter, Dr Katie Douglas, Dr Anna Fenton and Ms. Mayouri Sukhapure.

- I have read the Information sheet and understood the description and aims of the above-named project.

- I have had the opportunity to discuss the project with others in order to come to this decision.

- All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.

- I also understand that my participation in the project is entirely voluntary and that I may withdraw from the project at any time without any disadvantage. In the event that I withdraw from this study, all data collected from me will be destroyed and will not be included in the study.

- I understand the compensation provisions for this study.
• I know that as a participant, information pertaining to my medical records such as hormonal assessment/bodily fluids or blood, blood samples, cognitive and neuropsychological tests, completed questionnaires will be required for the project.
• I know that the questionnaires will explore my mood, thinking and feeling and that if the line of questioning develops in such a way that I feel hesitant or uncomfortable I may decline to answer any particular question(s), and/or may withdraw from the project without disadvantage of any kind.
• I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.
• I know that when the project is completed all personal identifying information will be removed from the paper records and electronic files which represent the data from the project, and that these will be placed in secure storage and kept for at least ten years.
• I understand that the results of the project may be published and be available in the University of Otago Library, but that I agree that any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study.

Signature of participant: ____________________________________________________________________________ Date: ____________________________________________________________________________

Signature and name of witness: ____________________________________________________________________________ Date: ____________________________________________________________________________

INVESTIGATORS NAME: ___________________________________________ DATE: __________

Signature: ___________________________________________
APPENDIX C

Flyers Used to Recruit Females with PCOS and Control Participants in the Current Study
Female participants needed for ‘Testosterone levels, Mood and Memory’ study

We, at the Department of Psychological medicine, University of Otago, Christchurch, are looking for females with untreated Polycystic Ovarian syndrome (PCOS) symptoms to assist in a study examining the relationship between sex hormones, mood, and memory in females. We will assess mood and cognition in a 1.5 hour testing session, for which you will be reimbursed with a $20 petrol voucher. If you are considered eligible to participate in the study you will be assessed by an endocrinologist who will recommend appropriate treatment and refer you back to your G.P.

If you are between 15-45 years of age and suspect you have PCOS (or have at least two of the following three symptoms) you will be eligible to participate in the study.

1) No other known endocrinological conditions
2) Irregular periods, positive scan of ovarian cysts
3) Symptoms such as abnormal hair growth, severe acne, weight gain issues, infertility, and family history of PCOS.
You must not have received treatment prior to this for PCOS.

Exclusion criteria:

1) No history of serious mental illness (e.g. Schizophrenia or Bipolar disorder)
2) No major neurological illness or severe head injury
3) Non-intact ovaries, pregnancy or menopause
Some of the things you will do as part of the study include:

1) Completing questionnaires which measure mental health symptoms
2) Doing tests on the computer, and some pen-and-paper tasks to assess learning, memory & emotion processing.

If you are interested in taking part, please call Mayouri:
Ph: 027 3076502, Email: sukma003@student.otago.ac.nz
VOLUNTEERS WANTED FOR SEX HORMONES, MOOD AND COGNITION STUDY

Our research team at the University of Otago, Christchurch, is looking for healthy females to assist in a study examining sex hormones, mood, cognitive function and emotion processing in females. We have examined a group of patients who have been diagnosed with PCOS (Polycystic Ovarian syndrome) and we are now recruiting healthy female volunteers between the ages of 16-40 years.

Participation includes completing questionnaires which measure mental health symptoms, doing tests on the computer as well as pen-and-paper tasks (which assess memory, attention and emotion processing) and getting a blood test done (to measure sex hormones levels).

The healthy volunteers we are recruiting must:
- Be between the ages of 16-40 years.
- Not have a history of serious mental illness (e.g. Schizophrenia or Bipolar disorder)
- Have their ovaries intact
- Not be pregnant or menopausal

You will receive compensation in the form of a $20 petrol voucher, to assist you in covering your travel related expenses.

If you would like more information or are interested in assisting with this research, please contact Mayouri Sukhapure at: sukma003@student.otago.ac.nz / 0273076502.

This study has been approved of by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email...
APPENDIX D

Polycystic Ovarian Syndrome Questionnaire (PCOS-Q)
Polycystic Ovarian Syndrome Questionnaire

Do you have any of the following conditions?

1. Excess hair growth on the face/back/chest/abdominal region
2. Irregular periods/Menstrual disturbances
3. Problems with weight management or weight gain issues?
4. Adult acne
5. Male pattern baldness/ acute hair loss
6. Have you ever had a scan of your ovaries?
7. Have you had problems getting pregnant or had miscarriages?
8. A brown-discoloured velvety texture to the skin especially near the neck or skin tags?
9. Is there a family history of PCOS?
10. Blood pressure
11. Clinician’s observations if any:
APPENDIX E

Letter of Ethical Approval for the Current Study
Dear Dr Porter,

I am again writing to you concerning your proposal entitled “The effect of abnormal androgen levels on mood symptoms, cognitive symptoms and depression in women based in Christchurch: A correlational study”, Ethics Committee reference number H14/047.

Thank you for your letter of 3rd April 2014 addressing the issues raised by the Committee.

The Committee appreciates the reconsideration of the proposed use of the three symptom measures noting that only two of the measures will now be used (one self-report (HADS) and an interview (QIDS)) as detailed in the original application. You note that the 90 minutes given for participants to complete the study would, in most cases, be more than sufficient time.

The Committee thanks you for attaching the Peer Review conducted by Associate Professor Chris Frampton and for the amendments made to the Information Sheet and Consent Form as requested.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor R Mulder  Head  Department of Psychological Medicine (ChCh)
APPENDIX F

Voucher Payment Agreement Form
Voucher Payment Agreement
Androgen and Mood Study

First payment

I, ______________________________ have received my first $20 petrol voucher to compensate for the time taken to be part of this study.

Signed: _______________________________ Date: -
____________________________________

Study investigator signature: _______________________________ Date: ______

Second payment

I, ______________________________ have received my second $20 petrol voucher to compensate for the time taken to be part of this study.

Signed: _______________________________ Date: -
____________________________________

Study investigator signature: _______________________________ Date: ______
APPENDIX G

National Adult Reading Test (NART) – 2nd Edition
### Recording Form for the National Adult Reading Test (NART) – 2nd Edition

<table>
<thead>
<tr>
<th>CHORD</th>
<th>IDYLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACHE</td>
<td>NAIVE</td>
</tr>
<tr>
<td>DEPOT</td>
<td>CATACOMB</td>
</tr>
<tr>
<td>AISLE</td>
<td>GAOLED</td>
</tr>
<tr>
<td>BOUQUET</td>
<td>THYME</td>
</tr>
<tr>
<td>PSALM</td>
<td>HEIR</td>
</tr>
<tr>
<td>CAPON</td>
<td>RADIX</td>
</tr>
<tr>
<td>DENY</td>
<td>ASSIGNATE</td>
</tr>
<tr>
<td>NAUSEA</td>
<td>HIATUS</td>
</tr>
<tr>
<td>DEBT</td>
<td>SUBTLE</td>
</tr>
<tr>
<td>COURTEOUS</td>
<td>PROCREATE</td>
</tr>
<tr>
<td>RAREFY</td>
<td>GIST</td>
</tr>
<tr>
<td>EQUIVOCAL</td>
<td>GOUGE</td>
</tr>
<tr>
<td>SUPERFLUOUS</td>
<td>PUIERPERAL</td>
</tr>
<tr>
<td>SIMILE</td>
<td>AVER</td>
</tr>
<tr>
<td>BANAL</td>
<td>GAUCHE</td>
</tr>
<tr>
<td>QUADRUPED</td>
<td>TOPIARY</td>
</tr>
<tr>
<td>CELLIST</td>
<td>LEVIATHAN</td>
</tr>
<tr>
<td>FAÇADE</td>
<td>BEATIFY</td>
</tr>
<tr>
<td>ZEALOT</td>
<td>PRELATE</td>
</tr>
<tr>
<td>DRACHM</td>
<td>SIDEREAL</td>
</tr>
<tr>
<td>AEON</td>
<td>DEMESNE</td>
</tr>
<tr>
<td>PLACEBO</td>
<td>SYNCOPE</td>
</tr>
<tr>
<td>ABSTEMIOUS</td>
<td>LABILE</td>
</tr>
<tr>
<td>DETENTE</td>
<td>CAMPANILE</td>
</tr>
</tbody>
</table>

**No. correct:** [ ]  **Number of errors:** [ ]  **Verbal IQ:** [ ]
# NART Conversion Table

<table>
<thead>
<tr>
<th>NART Errors</th>
<th>Predicted Verbal IQ</th>
<th>NART Errors</th>
<th>Predicted Verbal IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>127</td>
<td>26</td>
<td>98</td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>27</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>28</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>124</td>
<td>29</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>123</td>
<td>30</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>122</td>
<td>31</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>121</td>
<td>32</td>
<td>91</td>
</tr>
<tr>
<td>7</td>
<td>119</td>
<td>33</td>
<td>90</td>
</tr>
<tr>
<td>8</td>
<td>118</td>
<td>34</td>
<td>89</td>
</tr>
<tr>
<td>9</td>
<td>117</td>
<td>35</td>
<td>87</td>
</tr>
<tr>
<td>10</td>
<td>116</td>
<td>36</td>
<td>86</td>
</tr>
<tr>
<td>11</td>
<td>115</td>
<td>37</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td>114</td>
<td>38</td>
<td>84</td>
</tr>
<tr>
<td>13</td>
<td>113</td>
<td>39</td>
<td>83</td>
</tr>
<tr>
<td>14</td>
<td>111</td>
<td>40</td>
<td>82</td>
</tr>
<tr>
<td>15</td>
<td>110</td>
<td>41</td>
<td>81</td>
</tr>
<tr>
<td>16</td>
<td>109</td>
<td>42</td>
<td>80</td>
</tr>
<tr>
<td>17</td>
<td>108</td>
<td>43</td>
<td>78</td>
</tr>
<tr>
<td>18</td>
<td>107</td>
<td>44</td>
<td>77</td>
</tr>
<tr>
<td>19</td>
<td>106</td>
<td>45</td>
<td>76</td>
</tr>
<tr>
<td>20</td>
<td>105</td>
<td>46</td>
<td>75</td>
</tr>
<tr>
<td>21</td>
<td>103</td>
<td>47</td>
<td>74</td>
</tr>
<tr>
<td>22</td>
<td>102</td>
<td>48</td>
<td>73</td>
</tr>
<tr>
<td>23</td>
<td>101</td>
<td>49</td>
<td>72</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>50</td>
<td>70</td>
</tr>
</tbody>
</table>
CHORD ......................... harmonious combination of several different notes
ACHE .......................... prolonged dull pain
DEPOT .......................... storehouse, station, headquarters
AISLE .......................... passageway in a building
BOUQUET ....................... bunch of flowers
PSALM .......................... sacred song or hymn
CAPON .......................... castrated cock
DENY ........................... declare untrue or non-existent
NAUSEA ........................ feeling of sickness with an inclination to vomit
DEBT ........................... something that is owed
COURTEOUS .................... kind or considerate in manner
RAREFY ....................... refine (air), become less dense
EQUIVOCAL ..................... uncertain nature
NAÏVE ........................... innocent
CATACOMB .................... underground cemetery
GAOLED ........................ a jail
THYME .......................... culinary herb
HEIR ............................. legal successor or recipient of property/money/title
RADIX ........................... number of symbol used as the basis of a
                              numeration system - #10
ASSIGNATE ..................... appoint/assign a time/place/function
HIATUS .......................... gap in a series
SUBTLE .......................... evasive or mysterious, faint, delicate
PROCREATE ..................... process of reproduction
GIST ............................. essence of the matter
GOUGE .......................... groove made with a chisel
SUPERFLUOUS .................. not needed, more than enough
SIMILE .......................... compare one thing to another to illustrate
BANAL .......................... trite
QUADRUPED ........................ four-footed animal
CELLIST .......................... person who plays cello
FAÇADE .......................... face of a building, outward appearance
ZEALOT .......................... uncompromising or extreme partisan, fanatic
DRACHM .......................... measure 1/8 fluid oz
AEON .............................. an age
PLACEBO .......................... blank sample used as a control, a thing to calm/
                              humour but does not address
ABSTEMIOUS ........................ sparing, moderate in food and drink
DÉTENTE .......................... an easing of strained relations
IDYLL .............................. blissful period or scene
PUERPERAL ........................ due to childbirth
AVER .............................. assert, affirm
GAUGE .............................. lacking ease or grace
TOPIARY .......................... to do with clipped shrubs
LEVIATHAN ........................ sea monster, huge ship, person of great power
BEATIFY .......................... make happy, ecclesiastical (announce the
                              beatification of)
PRELATE .......................... high ecclesiastical dignitary
SIDEREAL .......................... determined by the stars
DEMENSE .......................... domain, territory, land owned by person or oneself
SYNCOPE .......................... shorten by omission of interior letter/s (pacifist)
LABILE .......................... unstable
CAMPINILE ........................ detached bell tower (as in Canterbury bells flower)
APPENDIX H

Demographic Questionnaires for the Current Study (Baseline and Follow-up)
Demographic Questionnaire

Androgen and Mood Study: Baseline

1. Age: 

2. D.O.B.: / / 

4. Years of secondary school education: 

5. Years of tertiary education: 

Ethnicity

6. Which ethnic group do you belong to? If you belong to multiple ethnic groups please tick appropriately (more than one box may be ticked):

[ ] New Zealand European
[ ] Maori
[ ] Samoan
[ ] Cook Island Maori
[ ] Tongan
[ ] Niuean
[ ] Chinese
[ ] Indian
[ ] Other (such as Dutch, Japanese, Tokelauan etc.) Please specify below:

........................................................................................................................................................................
Marital Status

7. What is your current marital status?

[ ] Single
[ ] Married
[ ] Widowed
[ ] Separated / Divorced
[ ] De facto

8. If currently in a relationship, how long (in years) have you been in your relationship?

9. How long (in years) is/was the longest intimate relationship you’ve had in your life?

Physical Health

10. Do you have any current physical illnesses (if so, what)?

........................................................................................................................................................................................................................................................................................................................................

11. (Current phase of menstruation cycle)

11.1 How long is your typical cycle?

........................................................................................................................................................................................................................................................................................................................................

11.2 What was the first day of your last period?

........................................................................................................................................................................................................................................................................................................................................
12. No of days since last period?

…………………………………………………………………………………………………………………………………………

13. Are you currently taking any medications (if so, what)?

………………………………………………………………………………………………………………………………………………….

14. Do you have any hearing or sight difficulties?

Y / N

Explain ........................................................................................................................................................................

15. Have you ever suffered from a significant brain injury?

Y / N

If so, was consciousness lost for more than 2 hours?

Y / N

16. Are you pregnant?

Y / N

Mental Illness

Have you ever been diagnosed or seen a doctor/psychologist for a mental health condition (e.g. Schizophrenia or Bipolar disorder?)

…………………………………………………………………………………………………………………………………………
Smoking

17. Do you smoke?
   
   If yes:
   
   How many cigarettes do you smoke per day?

   How long have you been smoking for?

   Number of pack years ( = packs / day x years)

Handedness

18. Are you left- or right-handed?
Demographic Questionnaire

Androgen and Mood Study: Follow-up

Physical Health

11. Do you have any current physical illnesses (if so, what)?

..................................................................................................................................................................................................................................................................................

11. No. of days in a typical cycle?

..................................................................................................................................................................................................................................................................................

12. No of days since last period?

..................................................................................................................................................................................................................................................................................

13. Are you currently taking any medications (if so, what)?

..................................................................................................................................................................................................................................................................................

Smoking

17. Do you smoke? Y / N
If yes:

How many cigarettes do you smoke per day?

How long have you been smoking for?

Number of pack years (\(= \text{packs/day} \times \text{years}\))
APPENDIX I

Mini International Neuropsychiatric Interview (MINI)
M.I.N.I.

MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW

English Version 5.0.0 DSM-IV

University of South Florida - Tampa

Hôpital de la Salpêtrière - Paris

© Copyright 1992-2006 Sheehan DV & Lecrubier Y

All rights reserved. No part of this document may be reproduced or transmitted in any form, or by any means, electronic or mechanical, including photocopying, or by any information storage or retrieval system, without permission in writing from Dr. Sheehan or Dr. Lecrubier. Researchers and clinicians working in nonprofit or publicly owned settings (including universities, nonprofit hospitals, and government institutions) may make copies of a M.I.N.I. instrument for their own clinical and research use.

DISCLAIMER

Our aim is to assist in the assessment and tracking of patients with greater efficiency and accuracy. Before action is taken on any data collected and processed by this program, it should be reviewed and interpreted by a licensed clinician.

This program is not designed or intended to be used in the place of a full medical and psychiatric evaluation by a qualified licensed physician – psychiatrist. It is intended only as a tool to facilitate accurate data collection and processing of symptoms elicited by trained personnel.

M.I.N.I. 5.0.0 (July 1, 2006)
<table>
<thead>
<tr>
<th>MODULES</th>
<th>MEETS TIME FRAME</th>
<th>CRITERIA</th>
<th>DSM-IV</th>
<th>ICD-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A MAJOR DEPRESSIVE EPISODE</td>
<td>Current (2 weeks)</td>
<td>☐</td>
<td>296.20-296.26 Single</td>
<td>F32.x</td>
</tr>
<tr>
<td></td>
<td>Recurrent</td>
<td>☐</td>
<td>296.30-296.36 Recurrent</td>
<td>F33.x</td>
</tr>
<tr>
<td>MDE WITH MELANCHOLIC FEATURES</td>
<td>Current (2 weeks)</td>
<td>☐</td>
<td>296.20-296.26 Single</td>
<td>F32.x</td>
</tr>
<tr>
<td></td>
<td>Optional</td>
<td></td>
<td>296.30-296.36 Recurrent</td>
<td>F33.x</td>
</tr>
<tr>
<td>B DYSTHYMIA</td>
<td>Current (Past 2 years)</td>
<td>☐</td>
<td>300.4</td>
<td>F34.1</td>
</tr>
<tr>
<td>C SUICIDALITY</td>
<td>Current (Past Month)</td>
<td>☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Risk: Low ☐ Medium ☐ High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D MANIC EPISODE</td>
<td>Current</td>
<td>☐</td>
<td>296.00-296.06</td>
<td>F30.x-F31.9</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYPOMANIC EPISODE</td>
<td>Current</td>
<td>☐</td>
<td>296.80-296.89</td>
<td>F31.8-F31.9/F34.0</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E PANIC DISORDER</td>
<td>Current (Past Month)</td>
<td>☐</td>
<td>300.01/300.21</td>
<td>F40.01-F41.0</td>
</tr>
<tr>
<td></td>
<td>Lifetime</td>
<td>☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F AGORAPHOBIA</td>
<td>Current</td>
<td>☐</td>
<td>300.22</td>
<td>F40.00</td>
</tr>
<tr>
<td>G SOCIAL PHOBIA (Social Anxiety Disorder)</td>
<td>Current (Past Month)</td>
<td>☐</td>
<td>300.23</td>
<td>F40.1</td>
</tr>
<tr>
<td>H OBSESSIVE-COMPULSIVE DISORDER</td>
<td>Current (Past Month)</td>
<td>☐</td>
<td>300.3</td>
<td>F42.8</td>
</tr>
<tr>
<td>I POSTTRAUMATIC STRESS DISORDER</td>
<td>Current (Past Month)</td>
<td>☐</td>
<td>309.81</td>
<td>F43.1</td>
</tr>
<tr>
<td>J ALCOHOL DEPENDENCE</td>
<td>Past 12 Months</td>
<td>☐</td>
<td>303.9</td>
<td>F10.2x</td>
</tr>
<tr>
<td></td>
<td>Past 12 Months</td>
<td>☐</td>
<td>305.00</td>
<td>F10.1</td>
</tr>
<tr>
<td>K SUBSTANCE DEPENDENCE (Non-alcohol)</td>
<td>Past 12 Months</td>
<td>☐</td>
<td>304.00-90/305.20-90</td>
<td>F11.1-F19.1</td>
</tr>
<tr>
<td></td>
<td>Past 12 Months</td>
<td>☐</td>
<td>304.00-90/305.20-90</td>
<td>F11.1-F19.1</td>
</tr>
<tr>
<td>L PSYCHOTIC DISORDERS</td>
<td>Lifetime</td>
<td>☐</td>
<td>295.10-295.90/297.1/297.3/293.81/293.82/293.89/298.8/298.9</td>
<td>F20.xx-F29</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>☐</td>
<td>296.24/296.34/296.44</td>
<td>F32.3/F33.3/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>296.24/296.34/296.44</td>
<td>F30.2/F31.2/F31.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F31.8/F31.9/F39</td>
</tr>
<tr>
<td>M ANOREXIA NERVOSA</td>
<td>Current (Past 3 Months)</td>
<td>☐</td>
<td>307.1</td>
<td>F50.0</td>
</tr>
<tr>
<td>N BULIMIA NERVOSA</td>
<td>Current (Past 3 Months)</td>
<td>☐</td>
<td>307.51</td>
<td>F50.2</td>
</tr>
<tr>
<td>ANOREXIA NERVOSA, BINGE EATING/PURGING TYPE</td>
<td>Current</td>
<td>☐</td>
<td>307.1</td>
<td>F50.0</td>
</tr>
<tr>
<td>Disorder</td>
<td>Timeframe</td>
<td>Code</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------</td>
<td>--------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>GENERALIZED ANXIETY DISORDER</td>
<td>Current (Past 6 Months)</td>
<td>300.02</td>
<td>F41.1</td>
<td></td>
</tr>
<tr>
<td>ANTISOCIAL PERSONALITY DISORDER</td>
<td>Lifetime</td>
<td>301.7</td>
<td>F60.2</td>
<td></td>
</tr>
</tbody>
</table>

Optional

Which problem troubles you the most? Indicate your response by checking the appropriate check box(es).
GENERAL INSTRUCTIONS

The M.I.N.I. was designed as a brief structured interview for the major Axis I psychiatric disorders in DSM-IV and ICD-10. Validation and reliability studies have been done comparing the M.I.N.I. to the SCID-P for DSM-III-R and the CIDI (a structured interview developed by the World Health Organization for lay interviewers for ICD-10). The results of these studies show that the M.I.N.I. has acceptably high validation and reliability scores, but can be administered in a much shorter period of time (mean 18.7 ± 11.6 minutes, median 15 minutes) than the above referenced instruments. It can be used by clinicians, after a brief training session. Lay interviewers require more extensive training.

INTERVIEW:
In order to keep the interview as brief as possible, inform the patient that you will conduct a clinical interview that is more structured than usual, with very precise questions about psychological problems which require a yes or no answer.

GENERAL FORMAT:
The M.I.N.I. is divided into modules identified by letters, each corresponding to a diagnostic category.
• At the beginning of each diagnostic module (except for psychotic disorders module), screening question(s) corresponding to the main criteria of the disorder are presented in a gray box.
• At the end of each module, diagnostic box(es) permit the clinician to indicate whether diagnostic criteria are met.

CONVENTIONS:
Sentences written in « normal font » should be read exactly as written to the patient in order to standardize the assessment of diagnostic criteria.

Sentences written in « CAPITALS » should not be read to the patient. They are instructions for the interviewer to assist in the scoring of the diagnostic algorithms.

Sentences written in « bold » indicate the time frame being investigated. The interviewer should read them as often as necessary. Only symptoms occurring during the time frame indicated should be considered in scoring the responses.

Answers with an arrow above them (↑) indicate that one of the criteria necessary for the diagnosis(es) is not met. In this case, the interviewer should go to the end of the module, circle « NO » in all the diagnostic boxes and move to the next module.

When terms are separated by a slash (/) the interviewer should read only those symptoms known to be present in the patient (for example, question H6).

Phrases in (parentheses) are clinical examples of the symptom. These may be read to the patient to clarify the question.

RATING INSTRUCTIONS:
All questions must be rated. The rating is done at the right of each question by circling either Yes or No. Clinical judgment by the rater should be used in coding the responses. The rater should ask for examples when necessary, to ensure accurate coding. The patient should be encouraged to ask for clarification on any question that is not absolutely clear.

The clinician should be sure that each dimension of the question is taken into account by the patient (for example, time frame, frequency, severity, and/or alternatives).

Symptoms better accounted for by an organic cause or by the use of alcohol or drugs should not be coded positive in the M.I.N.I. The M.I.N.I. Plus has questions that investigate these issues.

For any questions, suggestions, need for a training session, or information about updates of the M.I.N.I., please contact:

David V Sheehan, M.D., M.B.A.
University of South Florida College of Medicine
3515 East Fletcher Avenue
Tampa, FL, USA 33613-4788
tel : +1 813 974 4544; fax : +1 813 974 4575
e-mail : dsheehan@hsc.usf.edu
e-mail : hergueta@ext.jussieu.fr

Yves Lecrubier, M.D. / Thierry Hergueta, M.S.
INSERM U302
Hôpital de la Salpétrière
47, boulevard de l'Hôpital
F. 75651 PARIS, FRANCE
tel : +33 (0)1 42 16 16 59; fax : +33 (0)1 45 85 28 00

e-mail : dsheehan@hsc.usf.edu
e-mail : hergueta@ext.jussieu.fr
A. MAJOR DEPRESSIVE EPISODE

(MEANS: GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

<table>
<thead>
<tr>
<th>Question</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2 In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS A1 OR A2 CODED YES?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A3 Over the past two weeks, when you felt depressed or uninterested:

- a. Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by ±5% of body weight or ±8 lbs. or ±3.5 kgs., for a 160 lb./70 kg. person in a month)?
  - IF YES TO EITHER, CODE YES.

- b. Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?

- c. Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day?

- d. Did you feel tired or without energy almost every day?

- e. Did you feel worthless or guilty almost every day?

- f. Did you have difficulty concentrating or making decisions almost every day?

- g. Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead?

ARE 5 OR MORE ANSWERS (A1-A3) CODED YES?

IF PATIENT HAS CURRENT MAJOR DEPRESSIVE EPISODE CONTINUE TO A4, OTHERWISE MOVE TO MODULE B:

A4

- a. During your lifetime, did you have other episodes of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about?

- b. In between 2 episodes of depression, did you ever have an interval of at least 2 months, without any depression and any loss of interest?

* If patient has Major Depressive Episode, Current, use this information in coding the corresponding questions on page 5 (A6d, A6e).
MAJOR DEPRESSIVE EPISODE WITH MELANCHOLIC FEATURES (optional)

({=} MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF THE PATIENT CODES POSITIVE FOR A CURRENT MAJOR DEPRESSIVE EPISODE (A3 = YES), EXPLORE THE FOLLOWING:

| A5 | a | During the most severe period of the current depressive episode, did you lose almost completely your ability to enjoy nearly everything? | NO | YES |
| b | During the most severe period of the current depressive episode, did you lose your ability to respond to things that previously gave you pleasure, or cheered you up? | NO | YES |
| IF NO: | When something good happens does it fail to make you feel better, even temporarily? |

{=} IS EITHER A5a OR A5b CODED YES?

| A6 | Over the past two week period, when you felt depressed and uninterested: |
| a | Did you feel depressed in a way that is different from the kind of feeling you experience when someone close to you dies? | NO | YES |
| b | Did you feel regularly worse in the morning, almost every day? | NO | YES |
| c | Did you wake up at least 2 hours before the usual time of awakening and have difficulty getting back to sleep, almost every day? | NO | YES |
| d | IS A3c CODED YES (PSYCHOMOTOR RETARDATION OR AGITATION)? | NO | YES |
| e | IS A3a CODED YES FOR ANOREXIA OR WEIGHT LOSS? | NO | YES |
| f | Did you feel excessive guilt or guilt out of proportion to the reality of the situation? | NO | YES |

ARE 3 OR MORE A6 ANSWERS CODED YES

NO | YES
---|---
**Major Depressive Episode with Melancholic Features Current**
B. DYSTHYMIA

(MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF PATIENT’S SYMPTOMS CURRENTLY MEET CRITERIA FOR MAJOR DEPRESSIVE EPISODE, DO NOT EXPLORE THIS MODULE.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Have you felt sad, low or depressed most of the time for the last two years?</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>Was this period interrupted by your feeling OK for two months or more?</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>During this period of feeling depressed most of the time:</td>
</tr>
<tr>
<td>a</td>
<td>Did your appetite change significantly?</td>
</tr>
<tr>
<td>b</td>
<td>Did you have trouble sleeping or sleep excessively?</td>
</tr>
<tr>
<td>c</td>
<td>Did you feel tired or without energy?</td>
</tr>
<tr>
<td>d</td>
<td>Did you lose your self-confidence?</td>
</tr>
<tr>
<td>e</td>
<td>Did you have trouble concentrating or making decisions?</td>
</tr>
<tr>
<td>f</td>
<td>Did you feel hopeless?</td>
</tr>
<tr>
<td></td>
<td>ARE 2 OR MORE B3 ANSWERS CODED YES?</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B4</td>
<td>Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially, or in some other important way?</td>
</tr>
</tbody>
</table>
C. SUICIDALITY

In the past month did you:

C1  Suffer any accident?  NO  YES  Points 0
    IF NO TO C1, SKIP TO C2; IF YES, ASK C1a:
C1a Plan or intend to hurt yourself in that accident either passively or actively?  NO  YES  0
    IF NO TO C1a, SKIP TO C2; IF YES, ASK C1b:
C1b Did you intend to die as a result of this accident?  NO  YES  0

C2  Think that you would be better off dead or wish you were dead?  NO  YES  1

C3  Want to harm yourself or to hurt or to injure yourself?  NO  YES  2

C4  Think about suicide?  NO  YES  6

IF YES, ASK ABOUT THE INTENSITY AND FREQUENCY OF THE SUICIDAL IDEATION:

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occasionally</td>
<td></td>
</tr>
<tr>
<td>Often</td>
<td></td>
</tr>
</tbody>
</table>

Can you control these impulses and state that you will not act on them while in this program?  YES  8

Only score 8 points if response is NO.

C5  Have a suicide plan?  NO  YES  8

C6  Take any active steps to prepare to injure yourself or to prepare for a suicide attempt in which you expected or intended to die?  NO  YES  9

C7  Deliberately injure yourself without intending to kill yourself?  NO  YES  4

C8  Attempt suicide?
    Hoped to be rescued / survive  ❌
    Expected / intended to die    ❌  NO  YES  10

In your lifetime:

C9  Did you ever make a suicide attempt?  NO  YES  4

IS AT LEAST 1 OF THE ABOVE (EXCEPT C1) CODED YES?

IF YES, ADD THE TOTAL NUMBER OF POINTS FOR THE ANSWERS (C1-C9) CHECKED ‘YES’ AND SPECIFY THE LEVEL OF SUICIDE RISK AS INDICATED IN THE DIAGNOSTIC BOX:

SUICIDE RISK CURRENT

1-8 points Low  ❌
9-16 points Moderate  ❌
> 17 points High  ❌

MAKE ANY ADDITIONAL COMMENTS ABOUT YOUR ASSESSMENT OF THIS PATIENT’S CURRENT AND NEAR FUTURE SUICIDE RISK IN THE SPACE BELOW:
**D. (HYPO) MANIC EPISODE**

(☐ MEANS: GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

D1 a Have you ever had a period of time when you were feeling 'up' or 'high' or 'hyper' or so full of energy or full of yourself that you got into trouble, or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.)

IF PATIENT IS PUZZLED OR UNCLEAR ABOUT WHAT YOU MEAN BY 'UP' OR 'HIGH' OR 'HYPER', CLARIFY AS FOLLOWS: By 'up' or 'high' or 'hyper' I mean: having elated mood; increased energy; needing less sleep; having rapid thoughts; being full of ideas; having an increase in productivity, motivation, creativity, or impulsive behavior.

IF NO, CODE NO TO D1b: IF YES ASK:

b Are you currently feeling 'up' or 'high' or 'hyper' or full of energy?

D2 a Have you ever been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family? Have you or others noticed that you have been more irritable or over reacted, compared to other people, even in situations that you felt were justified?

IF NO, CODE NO TO D2b: IF YES ASK:

b Are you currently feeling persistently irritable?

Is D1a or D2a coded YES?

D3 IF D1b or D2b = YES: EXPLORE THE CURRENT AND THE MOST SYMPTOMATIC PAST EPISODE, OTHERWISE IF D1b AND D2b = NO: EXPLORE ONLY THE MOST SYMPTOMATIC PAST EPISODE

**During the times when you felt high, full of energy, or irritable did you:**

<table>
<thead>
<tr>
<th>Current Episode</th>
<th>Past Episode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a</strong> Feel that you could do things others couldn't do, or that you were an especially important person?</td>
<td>NO</td>
</tr>
<tr>
<td>IF YES, ASK FOR EXAMPLES. THE EXAMPLES ARE CONSISTENT WITH A DELUSIONAL IDEA.</td>
<td>☐ No ☐ Yes</td>
</tr>
<tr>
<td><strong>b</strong> Need less sleep (for example, feel rested after only a few hours sleep)?</td>
<td>NO</td>
</tr>
<tr>
<td><strong>c</strong> Talk too much without stopping, or so fast that people had difficulty understanding?</td>
<td>NO</td>
</tr>
<tr>
<td><strong>d</strong> Have racing thoughts?</td>
<td>NO</td>
</tr>
<tr>
<td><strong>e</strong> Become easily distracted so that any little interruption could distract you?</td>
<td>NO</td>
</tr>
<tr>
<td><strong>f</strong> Become so active or physically restless that others were worried about you?</td>
<td>NO</td>
</tr>
<tr>
<td><strong>g</strong> Want so much to engage in pleasurable activities that you ignored the risks or consequences (for example, spending sprees, reckless driving, or sexual indiscretions)?</td>
<td>NO</td>
</tr>
</tbody>
</table>
**D3 (SUMMARY):** ARE 3 OR MORE D3 ANSWERS CODED YES (OR 4 OR MORE IF D1a IS NO (IN RATING PAST EPISODE) AND D1b IS NO (IN RATING CURRENT EPISODE))?
RULE: Elation/expansiveness requires only three D3 symptoms while irritable mood alone requires 4 of the D3 symptoms.

VERIFY IF THE SYMPTOMS OCCURRED DURING THE SAME TIME PERIOD.

D4 Did these symptoms last at least a week and cause significant problems at home, at work, socially, or at school, or were you hospitalized for these problems?

THE EPISODE EXPLORED WAS A:

- HYPOMANIC EPISODE
- MANIC EPISODE
- HYPOMANIC MANIC EPISODE

IS D4 CODED NO?

SPECIFY IF THE EPISODE IS CURRENT OR PAST.

IS D4 CODED YES?

SPECIFY IF THE EPISODE IS CURRENT OR PAST.
E. PANIC DISORDER

(☐ means: Circle NO in E5, E6 and E7 and skip to F1)

<table>
<thead>
<tr>
<th>E</th>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 a</td>
<td>Have you, on more than one occasion, had spells or attacks when you <strong>suddenly</strong> felt anxious, frightened, uncomfortable or uneasy, even in situations where most people would not feel that way?</td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>Did the spells surge to a peak within 10 minutes of starting?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| E2 | At any time in the past, did any of those spells or attacks come on unexpectedly or occur in an unpredictable or unprovoked manner? | YES | NO |

| E3 | Have you ever had one such attack followed by a month or more of persistent concern about having another attack, or worries about the consequences of the attack or did you make a significant change in your behavior because of the attacks (e.g., shopping only with a companion, not wanting to leave your house, visiting the emergency room repeatedly, or seeing your doctor more frequently because of the symptoms? | YES | NO |

| E4 | During the worst spell that you can remember:                                                                                              | YES | NO |
|    | a. Did you have skipping, racing or pounding of your heart?                                                                                  |      | YES |
|    | b. Did you have sweating or clammy hands?                                                                                                  |      | YES |
|    | c. Were you trembling or shaking?                                                                                                           |      | YES |
|    | d. Did you have shortness of breath or difficulty breathing?                                                                               |      | YES |
|    | e. Did you have a choking sensation or a lump in your throat?                                                                               |      | YES |
|    | f. Did you have chest pain, pressure or discomfort?                                                                                         |      | YES |
|    | g. Did you have nausea, stomach problems or sudden diarrhea?                                                                               |      | YES |
|    | h. Did you feel dizzy, unsteady, lightheaded or faint?                                                                                    |      | YES |
|    | i. Did things around you feel strange, unreal, detached or unfamiliar, or did you feel outside of or detached from part or all of your body?   |      | YES |
|    | j. Did you fear that you were losing control or going crazy?                                                                                |      | YES |
|    | k. Did you fear that you were dying?                                                                                                       |      | YES |
|    | l. Did you have tingling or numbness in parts of your body?                                                                                |      | YES |
|    | m. Did you have hot flushes or chills?                                                                                                      |      | YES |

| E5 | Are both E3, and 4 or more E4 answers, coded YES?                                                                                         | YES | NO |
|    | If YES to E5, skip to E7.                                                                                                                  |      |    |

| E6 | If E5 = NO, Are any E4 answers coded YES?                                                                                                  | YES | NO |
|    | Then skip to F1.                                                                                                                           |      |    |
In the past month, did you have such attacks repeatedly (2 or more) followed by persistent concern about having another attack?

NO

YES

PANIC DISORDER
CURRENT
### F. AGORAPHOBIA

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F1</strong></td>
<td>Do you feel anxious or uneasy in places or situations where you might have a panic attack or the panic-like symptoms we just spoke about, or where help might not be available or escape might be difficult: like being in a crowd, standing in a line (queue), when you are alone away from home or alone at home, or when crossing a bridge, traveling in a bus, train or car?</td>
</tr>
<tr>
<td></td>
<td>NO YES</td>
</tr>
</tbody>
</table>

**IF F1 = NO, CIRCLE NO IN F2.**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F2</strong></td>
<td>Do you fear these situations so much that you avoid them, or suffer through them, or need a companion to face them?</td>
</tr>
<tr>
<td></td>
<td>NO YES</td>
</tr>
</tbody>
</table>

**IS F2 (CURRENT AGORAPHOBIA) CODED NO**

and

**IS E7 (CURRENT PANIC DISORDER) CODED YES?**

**NO**

**YES**

**PANIC DISORDER without Agoraphobia CURRENT**

**IS F2 (CURRENT AGORAPHOBIA) CODED YES**

and

**IS E7 (CURRENT PANIC DISORDER) CODED YES?**

**NO**

**YES**

**PANIC DISORDER with Agoraphobia CURRENT**

**IS F2 (CURRENT AGORAPHOBIA) CODED YES**

and

**IS E5 (PANIC DISORDER LIFETIME) CODED NO?**

**NO**

**YES**

**AGORAPHOBIA, CURRENT without history of Panic Disorder**
### G. SOCIAL PHOBIA (Social Anxiety Disorder)

(\(\checkmark\) MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO AND MOVE TO THE NEXT MODULE)

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 In the past month, were you fearful or embarrassed being watched, being the focus of attention, or fearful of being humiliated? This includes things like speaking in public, eating in public or with others, writing while someone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\checkmark\)

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2 Is this social fear excessive or unreasonable?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3 Do you fear these social situations so much that you avoid them or suffer through them?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G4 Do these social fears disrupt your normal work or social functioning or cause you significant distress?

**SUBTYPES**

Do you fear and avoid 4 or more social situations?

If YES Generalized social phobia (social anxiety disorder)

If NO Non-generalized social phobia (social anxiety disorder)

**NOTE TO INTERVIEWER:** PLEASE ASSESS WHETHER THE SUBJECT’S FEARS ARE RESTRICTED TO NON-GENERALIZED (“ONLY 1 OR SEVERAL”) SOCIAL SITUATIONS OR EXTEND TO GENERALIZED (“MOST”) SOCIAL SITUATIONS. “MOST” SOCIAL SITUATIONS IS USUALLY OPERATIONALIZED TO MEAN 4 OR MORE SOCIAL SITUATIONS, ALTHOUGH THE DSM-IV DOES NOT EXPLICITLY STATE THIS.

**EXAMPLES OF SUCH SOCIAL SITUATIONS** TYPICALLY INCLUDE INITIATING OR MAINTAINING A CONVERSATION, PARTICIPATING IN SMALL GROUPS, DATING, SPEAKING TO AUTHORITY FIGURES, ATTENDING PARTIES, PUBLIC SPEAKING, EATING IN FRONT OF OTHERS, URINATING IN A PUBLIC WASHROOM, ETC.
| H1 | In the past month, have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? (For example, the idea that you were dirty, contaminated or had germs, or fear of contaminating others, or fear of harming someone even though you didn't want to, or fearing you would act on some impulse, or fear or superstitions that you would be responsible for things going wrong, or obsessions with sexual thoughts, images or impulses, or hoarding, collecting, or religious obsessions.) | NO | YES | \( \downarrow \) \( \text{SKIP TO H4} \) |
| H2 | Did they keep coming back into your mind even when you tried to ignore or get rid of them? | NO | YES | \( \downarrow \) \( \text{SKIP TO H4} \) |
| H3 | Do you think that these obsessions are the product of your own mind and that they are not imposed from the outside? | NO | YES |
| H4 | In the past month, did you do something repeatedly without being able to things over and over, or repeating, collecting, arranging things, or other superstitious rituals? | NO | YES |
| \( \text{IS H3 OR H4 CODED YES?} \) | NO | YES |
| H5 | Did you recognize that either these obsessive thoughts or these compulsive behaviors were excessive or unreasonable? | NO | YES |
| H6 | Did these obsessive thoughts and/or compulsive behaviors significantly interfere with your normal routine, your work or school, your usual social activities, or relationships, or did they take more than one hour a day? | NO | YES | \( \text{O.C.D. CURRENT} \) |
### I. POSTTRAUMATIC STRESS DISORDER (optional)

(*{ means: go to the diagnostic box, circle NO, and move to the next module*)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Have you ever experienced or witnessed or had to deal with an extremely traumatic event that included actual or threatened death or serious injury to you or someone else?</td>
<td>⬇️</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>EXAMPLES OF TRAUMATIC EVENTS INCLUDE: SERIOUS ACCIDENTS, SEXUAL OR PHYSICAL ASSAULT, A TERRORIST ATTACK, BEING HELD HOSTAGE, KIDNAPPING, FIRE, DISCOVERING A BODY, SUDDEN DEATH OF SOMEONE CLOSE TO YOU, WAR, OR NATURAL DISASTER.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Did you respond with intense fear, helplessness or horror?</td>
<td>⬇️</td>
<td>NO</td>
</tr>
<tr>
<td>13</td>
<td>During the past month, have you re-experienced the event in a distressing way (such as, dreams, intense recollections, flashbacks or physical reactions)?</td>
<td>⬇️</td>
<td>NO</td>
</tr>
<tr>
<td>14</td>
<td>In the past month:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Have you avoided thinking about or talking about the event?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>b</td>
<td>Have you avoided activities, places or people that remind you of the event?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>c</td>
<td>Have you had trouble recalling some important part of what happened?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>d</td>
<td>Have you become much less interested in hobbies or social activities?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>e</td>
<td>Have you felt detached or estranged from others?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>f</td>
<td>Have you noticed that your feelings are numbed?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>g</td>
<td>Have you felt that your life will be shortened or that you will die sooner than other people?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td><strong>ARE 3 OR MORE 14 ANSWERS CODED YES?</strong></td>
<td>⬇️</td>
<td>NO</td>
</tr>
<tr>
<td>15</td>
<td>In the past month:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>Have you had difficulty sleeping?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>b</td>
<td>Were you especially irritable or did you have outbursts of anger?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>c</td>
<td>Have you had difficulty concentrating?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>d</td>
<td>Were you nervous or constantly on your guard?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>e</td>
<td>Were you easily startled?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td><strong>ARE 2 OR MORE 15 ANSWERS CODED YES?</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NO | YES
During the past month, have these problems significantly interfered with your work or social activities, or caused significant distress?
### J. ALCOHOL ABUSE AND DEPENDENCE

(Ì means: go to diagnostic boxes, circle no in both and move to the next module)

<table>
<thead>
<tr>
<th>J1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the past 12 months</strong>, have you had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>J2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the past 12 months:</strong></td>
</tr>
<tr>
<td><strong>a</strong> Did you need to drink more in order to get the same effect that you got when you first started drinking?</td>
</tr>
</tbody>
</table>
| **b** When you cut down on drinking did your hands shake, did you sweat or feel agitated? Did you drink to avoid these symptoms or to avoid being hungover, for example, "the shakes", sweating or agitation?  
  IF YES TO EITHER, CODE YES. | NO  YES |
| **c** During the times when you drank alcohol, did you end up drinking more than you planned when you started? | NO  YES |
| **d** Have you tried to reduce or stop drinking alcohol but failed? | NO  YES |
| **e** On the days that you drank, did you spend substantial time in obtaining alcohol, drinking, or in recovering from the effects of alcohol? | NO  YES |
| **f** Did you spend less time working, enjoying hobbies, or being with others because of your drinking? | NO  YES |
| **g** Have you continued to drink even though you knew that the drinking caused you health or mental problems? | NO  YES |

ARE 3 OR MORE J2 ANSWERS CODED YES?

* IF YES, SKIP J3 QUESTIONS, CIRCLE N/A IN THE ABUSE BOX AND MOVE TO THE NEXT DISORDER. DEPENDENCE PREEMPTS ABUSE.

<table>
<thead>
<tr>
<th>J3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the past 12 months:</strong></td>
</tr>
</tbody>
</table>
| **a** Have you been intoxicated, high, or hungover more than once when you had other responsibilities at school, at work, or at home? Did this cause any problems?  
  (Code yes only if this caused problems.) | NO  YES |
| **b** Were you intoxicated more than once in any situation where you were physically at risk, for example, driving a car, riding a motorbike, using machinery, boating, etc.? | NO  YES |
| **c** Did you have legal problems more than once because of your drinking, for example, an arrest or disorderly conduct? | NO  YES |
| **d** Did you continue to drink even though your drinking caused problems with your family or other people? | NO  YES |

ARE 1 OR MORE J3 ANSWERS CODED YES?

---

NO  N/A  YES

ALCOHOL ABUSE  
CURRENT

NO  YES*  

ALCOHOL DEPENDENCE  
CURRENT

432
K. NON-ALCOHOL PSYCHOACTIVE SUBSTANCE USE DISORDERS

(MEANS: GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

Now I am going to show you / read to you a list of street drugs or medicines.

K1. a. In the past 12 months, did you take any of these drugs more than once?

CIRCLE EACH DRUG TAKEN:


Cocaine: snorting, IV, freebase, crack, "speedball".

Narcotics: heroin, morphine, Dilaudid, opium, Demerol, methadone, codeine, Percodan, Darvon, OxyContin. Hallucinogens: LSD ("acid"), mescaline, peyote, PCP ("angel dust", "peace pill"), psilocybin, STP, "mushrooms", "ecstasy", MDA, MDMA, or ketamine ("special K").

Inhalants: "glue", ethyl chloride, "rush", nitrous oxide ("laughing gas"), amyl or butyl nitrate ("poppers").

Marijuana: hashish ("hash"), THC, "pot", "grass", "weed", "reefer".

Tranquilizers: Quaalude, Seconal ("reds"), Valium, Xanax, Librium, Ativan, Dalmane, Halcion, barbiturates, Miltown, GHB, Roofinol, "Roofies".

Miscellaneous: steroids, nonprescription sleep or diet pills. Any others?

SPECIFY MOST USED DRUG(S): ________________________________

CHECK ONE BOX

ONLY ONE DRUG / DRUG CLASS HAS BEEN USED

ONLY THE MOST USED DRUG CLASS IS INVESTIGATED.

EACH DRUG CLASS USED IS EXAMINED SEPARATELY (PHOTOCOPY K2 AND K3 AS NEEDED)

b SPECIFY WHICH DRUG/DRUG CLASS WILL BE EXPLORED IN THE INTERVIEW BELOW IF THERE IS CONCURRENT OR SEQUENTIAL POLYSUBSTANCE USE: ______________________________________________________________

K2 Considering your use of (NAME THE DRUG / DRUG CLASS SELECTED), in the past 12 months:

a Have you found that you needed to use more (NAME OF DRUG / DRUG CLASS SELECTED) to get the same effect that you did when you first started taking it?

b When you reduced or stopped using (NAME OF DRUG / DRUG CLASS SELECTED), did you have withdrawal symptoms (aches, shaking, fever, weakness, diarrhea, nausea, sweating, heart pounding, difficulty sleeping, or feeling agitated, anxious, irritable, or depressed)? Did you use any drug(s) to keep yourself from getting sick (withdrawal symptoms) or so that you would feel better?

IF YES TO EITHER, CODE YES.

c Have you often found that when you used (NAME OF DRUG / DRUG CLASS SELECTED), you ended up taking more than you thought you would?

d Have you tried to reduce or stop taking (NAME OF DRUG / DRUG CLASS SELECTED) but failed?

e On the days that you used (NAME OF DRUG / DRUG CLASS SELECTED), did you spend substantial time (>2 hours), obtaining, using or in recovering from the drug, or thinking about the drug?
Did you spend less time working, enjoying hobbies, or being with family or friends because of your drug use?  

NO  YES

Have you continued to use (name of drug / drug class selected), even though it caused you health or mental problems?  

NO  YES

ARE 3 OR MORE K2 ANSWERS CODED YES?

SPECIFY DRUG(S): ____________________________________________________________

* IF YES, SKIP K3 QUESTIONS, CIRCLE N/A IN THE ABUSE BOX FOR THIS SUBSTANCE AND MOVE TO THE NEXT DISORDER. DEPENDENCE PREEMPTS ABUSE.

Considering your use of (name the drug class selected), in the past 12 months:

K3 a Have you been intoxicated, high, or hungover from (name of drug / drug class selected) more than once, when you had other responsibilities at school, at work, or at home? Did this cause any problem?  

(NO  YES)

(CODE YES ONLY IF THIS CAUSED PROBLEMS.)

b Have you been high or intoxicated from (name of drug / drug class selected) more than once in any situation where you were physically at risk (for example, driving a car, riding a motorbike, using machinery, boating, etc.)?  

(NO  YES)

c Did you have legal problems more than once because of your drug use, for example, an arrest or disorderly conduct?  

(NO  YES)

d Did you continue to use (name of drug / drug class selected), even though it caused problems with your family or other people?  

(NO  YES)

ARE 1 OR MORE K3 ANSWERS CODED YES?

SPECIFY DRUG(S): ____________________________________________________________

NO N/A YES

SUBSTANCE ABUSE CURRENT

SUBSTANCE DEPENDENCE CURRENT
### L. PSYCHOTIC DISORDERS AND MOOD DISORDER WITH PSYCHOTIC FEATURES

Ask for an example of each question answered positively. Code **YES** only if the examples clearly show a distortion of thought or of perception or if they are not culturally appropriate. Before coding, investigate whether delusions qualify as "bizarre".

Delusions are "bizarre" if: clearly implausible, absurd, not understandable, and cannot derive from ordinary life experience. Hallucinations are scored "bizarre" if: a voice comments on the person's thoughts or behavior, or when two or more voices are conversing with each other.

Now I am going to ask you about unusual experiences that some people have.

<table>
<thead>
<tr>
<th>Question</th>
<th>NO</th>
<th>YES</th>
<th>BIZARRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 a Have you ever believed that people were spying on you, or that someone was plotting against you, or trying to hurt you?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NOTE</strong>: Ask for examples to rule out actual stalking.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b <strong>IF YES OR YES BIZARRE</strong>: do you currently believe these things?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2 a Have you ever believed that someone was reading your mind or could hear your thoughts, or that you could actually read someone's mind or hear what another person was thinking?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b <strong>IF YES OR YES BIZARRE</strong>: do you currently believe these things?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L3 a Have you ever believed that someone or some force outside of yourself put thoughts in your mind that were not your own, or made you act in a way that was not your usual self? Have you ever felt that you were possessed?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CLINICIAN</strong>: Ask for examples and discount any that are not psychotic.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b <strong>IF YES OR YES BIZARRE</strong>: do you currently believe these things?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L4 a Have you ever believed that you were being sent special messages through the TV, radio, or newspaper, or that a person you did not personally know was particularly interested in you?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b <strong>IF YES OR YES BIZARRE</strong>: do you currently believe these things?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L5 a Have your relatives or friends ever considered any of your beliefs strange or unusual?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INTERVIEWER</strong>: Ask for examples. Only code <strong>YES</strong> if the examples are <strong>CLEARLY</strong> delusional ideas not explored in questions L1 to L4, for example, somatic or religious delusions or delusions of grandiosity, jealousy, guilt, ruin or destitution, etc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b <strong>IF YES OR YES BIZARRE</strong>: do they currently consider your beliefs strange?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L6 a Have you ever heard things other people couldn't hear, such as voices?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HALLUCINATIONS ARE SCORED &quot;BIZARRE&quot; ONLY IF PATIENT ANSWERS YES TO THE FOLLOWING:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF <strong>YES</strong>: Did you hear a voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b <strong>IF YES OR YES BIZARRE TO L6a</strong>: have you heard these things in the past month?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HALLUCINATIONS ARE SCORED &quot;BIZARRE&quot; ONLY IF PATIENT ANSWERS YES TO THE FOLLOWING:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you hear a voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
L7  a  Have you ever had visions when you were awake or have you ever seen things other people couldn't see?  
CLINICIAN: CHECK TO SEE IF THESE ARE CULTURALLY INAPPROPRIATE.  

b  IF YES: have you seen these things in the past month?  

CLINICIAN'S JUDGMENT  

L8  b  IS THE PATIENT CURRENTLY EXHIBITING INCOHERENCE, DISORGANIZED SPEECH, OR MARKED LOOSENING OF ASSOCIATIONS?  

L9  b  IS THE PATIENT CURRENTLY EXHIBITING DISORGANIZED OR CATATONIC BEHAVIOR?  

L10  b  ARE NEGATIVE SYMPTOMS OF SCHIZOPHRENIA, E.G. SIGNIFICANT AFFECTIVE FLATTENING, POVERTY OF SPEECH (ALOGIA) OR AN INABILITY TO INITIATE OR PERSIST IN GOAL-DIRECTED ACTIVITIES (AVOLUTION), PROMINENT DURING THE INTERVIEW?  

L11  a  ARE 1 OR MORE « a » QUESTIONS FROM L1a TO L7a CODED YES OR YES BIZARRE AND IS EITHER:  

MAJOR DEPRESSIVE EPISODE, (CURRENT OR RECURRENT)  
OR  
MANIC OR HYPOMANIC EPISODE, (CURRENT OR PAST) CODED YES?  

IF NO TO L11 a, CIRCLE NO IN BOTH ‘MOOD DISORDER WITH PSYCHOTIC FEATURES’ DIAGNOSTIC BOXES AND MOVE TO L13.  

b  You told me earlier that you had period(s) when you felt (depressed/high/persistently irritable).  

Were the beliefs and experiences you just described (SYMPTOMS CODED YES FROM L1a TO L7a) restricted exclusively to times when you were feeling depressed/high/irritable?  

IF THE PATIENT EVER HAD A PERIOD OF AT LEAST 2 WEEKS OF HAVING THESE BELIEFS OR EXPERIENCES (PSYCHOTIC SYMPTOMS) WHEN THEY WERE NOT DEPRESSED/HIGH/IRRITABLE, CODE NO TO THIS DISORDER.  

IF THE ANSWER IS NO TO THIS DISORDER, ALSO CIRCLE NO TO L12 AND MOVE TO L13  

L12  a  ARE 1 OR MORE « b » QUESTIONS FROM L1b TO L7b CODED YES OR YES BIZARRE AND IS EITHER:  

MAJOR DEPRESSIVE EPISODE, (CURRENT)  
OR  
MANIC OR HYPOMANIC EPISODE, (CURRENT) CODED YES?  

IF THE ANSWER IS YES TO THIS DISORDER (LIFETIME OR CURRENT), CIRCLE NO TO L13 AND L14 AND MOVE TO THE NEXT MODULE.
L13 ARE 1 OR MORE « b » QUESTIONS FROM L1b TO L6b, CODED YES BIZARRE?

OR

ARE 2 OR MORE « b » QUESTIONS FROM L1b TO L10b, CODED YES (RATHER THAN YES BIZARRE)?

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?

L14 IS L13 CODED YES

OR

ARE 1 OR MORE « a » QUESTIONS FROM L1a TO L6a, CODED YES BIZARRE?

OR

ARE 2 OR MORE « a » QUESTIONS FROM L1a TO L7a, CODED YES (RATHER THAN YES BIZARRE)

AND DID AT LEAST TWO OF THE PSYCHOTIC SYMPTOMS OCCUR DURING THE SAME 1 MONTH PERIOD?
M. ANOREXIA NERVOSA

(M. MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

<table>
<thead>
<tr>
<th>M1 a How tall are you?</th>
<th>ft</th>
<th>in.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lbs.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the past 3 months:

M2 In spite of this low weight, have you tried not to gain weight? NO YES

M3 Have you intensely feared gaining weight or becoming fat, even though you were underweight? NO YES

M4 a Have you considered yourself too big / fat or that part of your body was too big / fat? NO YES

 b Has your body weight or shape greatly influenced how you felt about yourself? NO YES

c Have you thought that your current low body weight was normal or excessive? NO YES

M5 ARE 1 OR MORE ITEMS FROM M4 CODED YES? NO YES

M6 FOR WOMEN ONLY: During the last 3 months, did you miss all your menstrual periods when they were expected to occur (when you were not pregnant)? NO YES

FOR WOMEN: ARE M5 AND M6 CODED YES?  

FOR MEN: IS M5 CODED YES?

HEIGHT / WEIGHT TABLE CORRESPONDING TO A BMI THRESHOLD OF 17.5 KG/M²

| Height/Weight | ft/in | 4'9  | 4'10 | 4'11 | 5'0  | 5'1  | 5'2  | 5'3  | 5'4  | 5'5  | 5'6  | 5'7  | 5'8  | 5'9  | 5'10 |
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| lbs.          | 81   | 84   | 87   | 89   | 92   | 96   | 99   | 102  | 105  | 108  | 112  | 115  | 118  | 122  |
| cm.           | 145  | 147  | 150  | 152  | 155  | 158  | 160  | 163  | 165  | 168  | 170  | 173  | 175  | 178  |
| kgs           | 37   | 38   | 39   | 41   | 42   | 43   | 44   | 46   | 48   | 49   | 51   | 52   | 54   | 55   |

<table>
<thead>
<tr>
<th>Height/Weight</th>
<th>ft/in</th>
<th>5'11</th>
<th>6'0</th>
<th>6'1</th>
<th>6'2</th>
<th>6'3</th>
</tr>
</thead>
<tbody>
<tr>
<td>lbs.</td>
<td>125</td>
<td>129</td>
<td>132</td>
<td>136</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>cm.</td>
<td>180</td>
<td>183</td>
<td>185</td>
<td>188</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>kgs</td>
<td>57</td>
<td>59</td>
<td>60</td>
<td>62</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

The weight thresholds above are calculated using a body mass index (BMI) equal to or below 17.5 kg/m² for the patient's height. This is the threshold guideline below which a person is deemed underweight by the DSM-IV and the ICD-10 Diagnostic Criteria for Research for Anorexia Nervosa.
### N. BULIMIA NERVOSA

(☐ MEANS: GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

<table>
<thead>
<tr>
<th>Q</th>
<th>Question</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>In the past three months, did you have eating binges or times when you ate a very large amount of food within a 2-hour period?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>N2</td>
<td>In the last 3 months, did you have eating binges as often as twice a week?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>N3</td>
<td>During these binges, did you feel that your eating was out of control?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>N4</td>
<td>Did you do anything to compensate for, or to prevent a weight gain from these binges, like vomiting, fasting, exercising or taking laxatives, enemas, diuretics (fluid pills), or other medications?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>N5</td>
<td>Does your body weight or shape greatly influence how you feel about yourself?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>N6</td>
<td>DO THE PATIENT’S SYMPTOMS MEET CRITERIA FOR ANOREXIA NERVOSA?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td><strong>INTERVIEWER:</strong> WRITE IN THE ABOVE PARENTHESIS THE THRESHOLD WEIGHT FOR THIS PATIENT’S HEIGHT FROM THE HEIGHT / WEIGHT TABLE IN THE ANOREXIA NERVOSA MODULE.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N7</td>
<td>Do these binges occur only when you are under (___ lbs./kgs.)?</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

IS N5 CODED YES AND IS EITHER N6 OR N7 CODED NO?

IS N7 CODED YES?

---

**BULIMIA NERVOSA CURRENT**

**ANOREXIA NERVOSA**

*Binge Eating/Purging Type*
# O. GENERALIZED ANXIETY DISORDER

($) MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE

<table>
<thead>
<tr>
<th>O1</th>
<th>a Have you worried excessively or been anxious about several things over the past 6 months?</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b Are these worries present most days?</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>IS THE PATIENT’S ANXIETY RESTRICTED EXCLUSIVELY TO, OR BETTER EXPLAINED BY, ANY DISORDER PRIOR TO THIS POINT?</td>
<td>YES</td>
</tr>
</tbody>
</table>

| O2   | Do you find it difficult to control the worries or do they interfere with your ability to focus on what you are doing? | YES |

| O3   | FOR THE FOLLOWING, CODE NO IF THE SYMPTOMS ARE CONFINED TO FEATURES OF ANY DISORDER EXPLORED PRIOR TO THIS POINT. | YES |

When you were anxious over the past 6 months, did you, most of the time:

<table>
<thead>
<tr>
<th>a</th>
<th>Feel restless, keyed up or on edge?</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>Feel tense?</td>
<td>YES</td>
</tr>
<tr>
<td>c</td>
<td>Feel tired, weak or exhausted easily?</td>
<td>YES</td>
</tr>
<tr>
<td>d</td>
<td>Have difficulty concentrating or find your mind going blank?</td>
<td>YES</td>
</tr>
<tr>
<td>e</td>
<td>Feel irritable?</td>
<td>YES</td>
</tr>
<tr>
<td>f</td>
<td>Have difficulty sleeping (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?</td>
<td>YES</td>
</tr>
</tbody>
</table>

ARE 3 OR MORE O3 ANSWERS CODED YES?

| NO | YES |

GENERALIZED ANXIETY DISORDER CURRENT
P. ANTISOCIAL PERSONALITY DISORDER (optional)

(Ε means: go to the diagnostic box and circle NO.)

P1 Before you were 15 years old, did you:

<table>
<thead>
<tr>
<th>Question</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>a repeatedly skip school or run away from home overnight?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b repeatedly lie, cheat, &quot;con&quot; others, or steal?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c start fights or bully, threaten, or intimidate others?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d deliberately destroy things or start fires?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e deliberately hurt animals or people?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f force someone to have sex with you?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ARE 2 OR MORE P1 ANSWERS CODED YES?  

DO NOT CODE YES TO THE BEHAVIORS BELOW IF THEY ARE EXCLUSIVELY POLITICALLY OR RELIGIOUSLY MOTIVATED.

P2 Since you were 15 years old, have you:

<table>
<thead>
<tr>
<th>Question</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>a repeatedly behaved in a way that others would consider irresponsible, like failing to pay for things you owed, deliberately being impulsive or deliberately not working to support yourself?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b done things that are illegal even if you didn't get caught (for example, destroying property, shoplifting, stealing, selling drugs, or committing a felony)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c been in physical fights repeatedly (including physical fights with your spouse or children)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d often lied or &quot;conned&quot; other people to get money or pleasure, or lied just for fun?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e exposed others to danger without caring?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f felt no guilt after hurting, mistreating, lying to, or stealing from others, or after damaging property?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ARE 3 OR MORE P2 QUESTIONS CODED YES?  

ANTISOCIAL PERSONALITY DISORDER LIFETIME

NO YES

THIS CONCLUDES THE INTERVIEW
REFERENCES


**M.I.N.I. 4.6/5.0, M.I.N.I. Plus 4.6/5.0 Translations**

<table>
<thead>
<tr>
<th>Translations</th>
<th>M.I.N.I. 4.4 or earlier versions</th>
<th>M.I.N.I. 4.6 and M.I.N.I. Screen 5.0:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afrikaans</td>
<td>R. Emsley</td>
<td>W. Maartens</td>
</tr>
<tr>
<td>Arabic</td>
<td>P. Bech</td>
<td>O. Osman, E. Al-Radi</td>
</tr>
<tr>
<td>Bengali</td>
<td>E. Griez, K. Shruers, T. Overbeek, K. Demyttenaere</td>
<td>H. Banerjee, A. Banerjee</td>
</tr>
<tr>
<td>Braille (English)</td>
<td>D. Sheehan, J. Janavs, R. Baker, K. Harnett-Sheehan, E. Knapp, M. Sheehan</td>
<td>P. Amorim</td>
</tr>
<tr>
<td>Brazilian Portuguese</td>
<td>P. Amorim</td>
<td>L. Carroll, Y-J. Lee, Y-S. Chen, C-C. Chen, C-Y. Liu,</td>
</tr>
<tr>
<td>Bulgarian</td>
<td>L.G. Hranov</td>
<td>P. Zvolsky</td>
</tr>
<tr>
<td>English</td>
<td>G. Pedersen, S. Blomhoff</td>
<td>M. Heikkinen, M. Lijestrom, O. Tuominen</td>
</tr>
<tr>
<td>German</td>
<td>I. v. Denffer, M. Achenheil, R. Dietz-Bauer</td>
<td>T. Calligas, S. Beratis</td>
</tr>
<tr>
<td>Greek</td>
<td>S. Beratis</td>
<td>M. Patel, B. Patel, Organon</td>
</tr>
<tr>
<td>Gujarati</td>
<td>J. Zohar, Y. Sasson</td>
<td>R. Barda, I. Levinson, A. Aviv</td>
</tr>
<tr>
<td>Hebrew</td>
<td>I. Bitter, J. Balazs</td>
<td>C. Mittal, K. Batra, S. Gambhir, Organon</td>
</tr>
<tr>
<td>Hungarian</td>
<td>I. Bitter, J. Balazs</td>
<td>I. Bitter, J. Balazs</td>
</tr>
<tr>
<td>Icelandic</td>
<td>I. Bitter, J. Balazs</td>
<td>J.G. Stefansson</td>
</tr>
<tr>
<td>Kannada</td>
<td></td>
<td>K.S. Oh and Korean Academy of Anxiety Disorders, V. Janavs, J. Janavs</td>
</tr>
<tr>
<td>Korean</td>
<td>V. Janavs, I. Nagobads</td>
<td>A. Bacevicius, Organon</td>
</tr>
<tr>
<td>Latvian</td>
<td></td>
<td>K.A. Leiknes, U. Malt, E. Malt, S. Leganger</td>
</tr>
<tr>
<td>Lithuanian</td>
<td></td>
<td>M. Masiak, E. Jasiak</td>
</tr>
<tr>
<td>Malayalam</td>
<td></td>
<td>P. Amorim, T. Guteres</td>
</tr>
<tr>
<td>Marathi</td>
<td></td>
<td>A. Gahunia, S. Gambhir</td>
</tr>
<tr>
<td>Norwegian</td>
<td>G. Pedersen, S. Blomhoff</td>
<td>O. Driga</td>
</tr>
<tr>
<td>Polish</td>
<td>M. Masiak, E. Jasiak</td>
<td>A. Bystritsky, E. Selivra, M. Bystritsky, L. Shumyak, I. Timotijevic</td>
</tr>
<tr>
<td>Portuguese</td>
<td>P. Amorim</td>
<td>I. Timotijevic</td>
</tr>
<tr>
<td>Punjabi</td>
<td></td>
<td>K. Ketlogetsw</td>
</tr>
<tr>
<td>Romanian</td>
<td></td>
<td>M. Kocmur, M. Kocmur</td>
</tr>
<tr>
<td>Russian</td>
<td></td>
<td>L. Ferrando, L. Franco-Alfonso, M. Soto, J. Bobes-</td>
</tr>
<tr>
<td>M. Klisinska.</td>
<td></td>
<td>C. Allgulander, H. Agren M. Waern, A. Brimse, Organon</td>
</tr>
<tr>
<td>Serbian</td>
<td>I. Timotijevic</td>
<td>Organon</td>
</tr>
<tr>
<td>Setswana</td>
<td></td>
<td>Organon</td>
</tr>
<tr>
<td>Slovenian</td>
<td></td>
<td>Organon</td>
</tr>
<tr>
<td>Spanish</td>
<td>L. Ferrando, J. Bobes-Garcia, J. Gilbert-Rahola, Y. Lecrubier</td>
<td>M. Kocmur, M. Kocmur</td>
</tr>
<tr>
<td>Garcia, O. Soto, L. Franco, G. Heinze, C. Santana,</td>
<td>L. Ferrando, L. Franco-Alfonso, M. Soto, J. Bobes-</td>
<td></td>
</tr>
<tr>
<td>R. Hidalgo</td>
<td></td>
<td>C. Allgulander, H. Agren M. Waern, A. Brimse, Organon</td>
</tr>
<tr>
<td>Swedish</td>
<td>M. Waern, S. Andersch, M. Humble</td>
<td>Organon</td>
</tr>
<tr>
<td>M. Humble.</td>
<td></td>
<td>Organon</td>
</tr>
<tr>
<td>Tamil</td>
<td></td>
<td>Organon</td>
</tr>
<tr>
<td>Telugu</td>
<td></td>
<td>Organon</td>
</tr>
</tbody>
</table>

442
A validation study of this instrument was made possible, in part, by grants from SmithKline Beecham and the European Commission. The authors are grateful to Dr. Pauline Powers for her advice on the modules on Anorexia Nervosa and Bulimia.
APPENDIX J

Depression Rating Scales used in the Current Study- Hospital Anxiety and Depression Rating Scale (HADS) and Quick Inventory of Depressive Symptomatology (QIDS)
Hospital Anxiety and Depression Scale (HADS)

Patients are asked to choose one response from the four given for each interview. They should give an immediate response and be dissuaded from thinking too long about their answers. The questions relating to anxiety are marked "A", and to depression "D". The score for each answer is given in the right column. Instruct the patient to answer how it currently describes their feelings.

<table>
<thead>
<tr>
<th>A</th>
<th>I feel tense or 'wound up':</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Most of the time</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>A lot of the time</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>From time to time, occasionally</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>I still enjoy the things I used to enjoy:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definitely as much</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Not quite so much</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Only a little</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hardly at all</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>I get a sort of frightened feeling as if something awful is about to happen:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very definitely and quite badly</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Yes, but not too badly</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>A little, but it doesn't worry me</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Not at all</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>I can laugh and see the funny side of things:</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>As much as I always could</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Not quite so much now</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Definitely not so much now</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Not at all</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>Worrying thoughts go through my mind:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A great deal of the time</td>
<td>3</td>
</tr>
<tr>
<td>A lot of the time</td>
<td>2</td>
</tr>
<tr>
<td>From time to time, but not too often</td>
<td>1</td>
</tr>
<tr>
<td>Only occasionally</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>I feel cheerful:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>3</td>
</tr>
<tr>
<td>Not often</td>
<td>2</td>
</tr>
<tr>
<td>Sometimes</td>
<td>1</td>
</tr>
<tr>
<td>Most of the time</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>I can sit at ease and feel relaxed:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitely</td>
<td>0</td>
</tr>
<tr>
<td>Usually</td>
<td>1</td>
</tr>
<tr>
<td>Not Often</td>
<td>2</td>
</tr>
<tr>
<td>Not at all</td>
<td>3</td>
</tr>
</tbody>
</table>
D  I feel as if I am slowed down:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nearly all the time</td>
<td>3</td>
</tr>
<tr>
<td>Very often</td>
<td>2</td>
</tr>
<tr>
<td>Sometimes</td>
<td>1</td>
</tr>
<tr>
<td>Not at all</td>
<td>0</td>
</tr>
</tbody>
</table>

A  I get a sort of frightened feeling like 'butterflies' in the stomach:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>0</td>
</tr>
<tr>
<td>Occasionally</td>
<td>1</td>
</tr>
<tr>
<td>Quite Often</td>
<td>2</td>
</tr>
<tr>
<td>Very Often</td>
<td>3</td>
</tr>
</tbody>
</table>

D  I have lost interest in my appearance:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitely</td>
<td>3</td>
</tr>
<tr>
<td>I don't take as much care as I should</td>
<td>2</td>
</tr>
<tr>
<td>I may not take quite as much care</td>
<td>1</td>
</tr>
<tr>
<td>I take just as much care as ever</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>I feel restless as I have to be on the move:</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Very much indeed: 3</td>
</tr>
<tr>
<td></td>
<td>Quite a lot: 2</td>
</tr>
<tr>
<td></td>
<td>Not very much: 1</td>
</tr>
<tr>
<td></td>
<td>Not at all: 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>I look forward with enjoyment to things:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>As much as I ever did: 0</td>
</tr>
<tr>
<td></td>
<td>Rather less than I used to: 1</td>
</tr>
<tr>
<td></td>
<td>Definitely less than I used to: 2</td>
</tr>
<tr>
<td></td>
<td>Hardly at all: 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>I get sudden feelings of panic:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very often indeed: 3</td>
</tr>
<tr>
<td></td>
<td>Quite often: 2</td>
</tr>
<tr>
<td></td>
<td>Not very often: 1</td>
</tr>
<tr>
<td></td>
<td>Not at all: 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>I can enjoy a good book or radio or TV program:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Often: 0</td>
</tr>
<tr>
<td></td>
<td>Sometimes: 1</td>
</tr>
<tr>
<td></td>
<td>Not often: 2</td>
</tr>
<tr>
<td></td>
<td>Very seldom: 3</td>
</tr>
</tbody>
</table>
Scoring (add the As = Anxiety. Add the Ds = Depression). The norms below will give you an idea of the level of Anxiety and Depression.

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>Normal</td>
</tr>
<tr>
<td>8-10</td>
<td>Borderline abnormal</td>
</tr>
<tr>
<td>11-21</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

Reference:

_Zigmond and Snaith (1983)_
QUICK INVENTORY OF DEPRESSIVE SYMPTOMATOLOGY (QIDS)

Clinician Instructions: In making each rating, consider the frequency, duration, and intensity/severity of the symptom. The degree of functional impairment caused by the symptom may be important in the ratings of some items, but not all. If patient denies ever experiencing euthymic mood, ask them to compare the last week to a time that they felt their best or to compare to what they would consider to be a satisfactory level of functioning.

INTRODUCTION:

“I would like to ask you some questions about the past week, the last 7 days.”

1) How have you been sleeping in the past week? Have you had trouble falling asleep when you go to bed? Right after you go to bed, how long does it take you to fall asleep? How many days in the past week have you had trouble falling asleep?

2) During the past week, have you been waking in the middle of the night?
   IF YES: How often have you been waking up? How long do you stay awake? Do you get out of bed? What do you do? Are you able to fall right back to sleep? Have you felt your sleep has been restless or disturbed some nights?

3) What time have you been waking up in the past week? With or without an alarm? Have you been waking earlier than you want to or need to? How much earlier than is normal for you? How many days? Are you able to go back to sleep?

4) How many hours on average have you been sleeping in a 24-hour period in the past week? Does that include naps? Is that a normal amount for you? What is the longest you've slept in a 24-hour period in the last week?

5) How would you describe your mood in the past week? Have you been feeling down or depressed? Sad? In the past week, how much of the time have you felt _____? Every day? All day?

6) How has your appetite been in the last week compared to your usual appetite? Have you had to force yourself to eat? Have others urged or reminded you to eat? In the past week, how often have you eaten? Every day? When you do eat, have you noticed that you eat less than usual?

7) Have you found yourself eating more than usual? Every day? Have you noticed you eat more at meals? Have you noticed you are snacking or eating more in between meals? Have you felt driven to eat? Have you had eating binges?
8/9) Have you noticed any change in your weight? Are your clothes fitting differently than usual? How much has your weight changed in the past 2 weeks?

10) Have you noticed any problems with your concentration in the past week? Have you been able to focus on what you have been doing (like reading or watching TV)? In the past week, have you noticed having problems making decisions? Were minor decisions more difficult than usual to make (what to wear, eat, or watch on TV)? In the past week, how often have you had problems with________?

11) In the past week, have you been feeling especially critical of yourself? Have you been feeling like you have done things wrong, let others down, or caused problems for others? IF YES: What have your thoughts been? In the past week, have you felt worthless? IF YES: How often have you felt worthless? Everyday? All day?

12) In the past week, have you felt that life was not worth living, or that you’d be better off dead? What about thoughts of hurting or killing yourself? IF YES: How often do you think about________? When you think about________________ , how long do you think about it? What have you thought about? Do you have a plan? Have you done anything to hurt yourself? What stops you? (THOROUGHLY ASSESS SUICIDE POTENTIAL.)

13) How have you been spending your time this past week? How would you describe your level of interest and motivation to complete daily activities? Have you felt interested in doing those things or do you feel you have to push yourself to do them? Have you stopped doing anything you used to do? IF YES: Is there anything you look forward to doing? Have you been able to maintain your personal hygiene?

14) How has your energy level been this past week? Have you been tired all the time? IF NO: Have you noticed you tire more easily than usual? This week have you had backaches, headaches, aches, or heaviness in your head or limbs? Has your lack of energy interfered with your ability to carry out most of your usual daily activities?

15) Have you felt slowed down in your thinking, speaking, or movement in the past week? Have others commented on this? How many days in the past week have you felt___? When you feel___, how long does it last?

16) Have you noticed feeling fidgety or speeded up during the past week? Have you found yourself unable to stay seated or needing to move around more than is typical for you? How often do you feel___________________________? How long does it last?
QUICK INVENTORY OF DEPRESSIVE SYMPTOMATOLOGY (CLINICIAN-RATED)
(QIDS-C)

NAME: ____________________________ TODAY’S DATE: __________

Please circle one response to each item that best describes the patient for the last seven days.

1. Sleep Onset Insomnia:
   0 Never takes longer than 30 minutes to fall asleep.
   1 Takes at least 30 minutes to fall asleep, less than half the time.
   2 Takes at least 30 minutes to fall asleep, more than half the time.
   3 Takes more than 60 minutes to fall asleep, more than half the time.

2. Mid-Nocturnal Insomnia:
   0 Does not wake up at night.
   1 Restless, light sleep with few awakenings.
   2 Wakes up at least once a night, but goes back to sleep easily.
   3 Awakens more than once a night and stays awake for 20 minutes or more, more than half the time.

3. Early Morning Insomnia:
   0 Less than half the time, awakens no more than 30 minutes before necessary.
   1 More than half the time, awakens more than 30 minutes before need be.
   2 Awakens at least one hour before need be, more than half the time.
   3 Awakens at least two hours before need be, more than half the time.

4. Hypersomnia:
   0 Sleeps no longer than 7-8 hours/night, without naps.
   1 Sleeps no longer than 10 hours in a 24 hour period (include naps).
   2 Sleeps no longer than 12 hours in a 24 hour period (include naps).
   3 Sleeps longer than 12 hours in a 24 hour period (include naps).

5. Mood (Sad):
   0 Does not feel sad.
   1 Feels sad less than half the time.
   2 Feels sad more than half the time.
   3 Feels intensely sad virtually all the time.

6. Appetite (Decreased):
   0 No change from usual appetite.
   1 Eats somewhat less often and/or lesser amounts than usual.
   2 Eats much less than usual and only with personal effort.
   3 Eats rarely within a 24-hour period, and only with extreme personal effort or with persuasion by others.

7. Appetite (Increased):
   0 No change from usual appetite.
   1 More frequently feels a need to eat than usual.
   2 Regularly eats more often and/or greater amounts than usual.
   3 Feels driven to overeat at and between meals.

8. Weight (Decrease) Within the Last Two Weeks:
   0 Has experienced no weight change.
   1 Feels as if some slight weight loss occurred.
   2 Has lost 2 pounds or more.
   3 Has lost 5 pounds or more.

9. Weight (Increase) Within the Last Two Weeks:
   0 Has experienced no weight change.
   1 Feels as if some slight weight gain has occurred.
   2 Has gained 2 pounds or more.
   3 Has gained 5 pounds or more.

Enter the highest score on any 1 of the 4 appetite/weight change items (6–9 above) _____

10. Concentration/Decision Making:
    0 No change in usual capacity to concentrate and decide.
    1 Occasionally feels indecisive or notes that attention often wanders.
    2 Most of the time struggles to focus attention or make decisions.
    3 Cannot concentrate well enough to read or cannot make even minor decisions.
11. Outlook (Self):
   0  Sees self as equally worthwhile and deserving as others.
   1  Is more self-blaming than usual.
   2  Largely believes that he/she causes problems for others.
   3  Ruminates over major and minor defects in self.

12. Suicidal Ideation:
   0  Does not think of suicide or death.
   1  Feels life is empty or is not worth living.
   2  Thinks of suicide/death several times a week for several minutes.
   3  Thinks of suicide/death several times a day in depth, or has made specific plans, or attempted suicide.

13. Involvement:
   0  No change from usual level of interest in other people and activities.
   1  Notices a reduction in former interests/activities.
   2  Finds only one or two former interests remain.
   3  Has virtually no interest in formerly pursued activities.

14. Energy/Fatiguability:
   0  No change in usual level of energy.
   1  Tires more easily than usual.
   2  Makes significant personal effort to initiate or maintain usual daily activities.
   3  Unable to carry out most of usual daily activities due to lack of energy.

15. Psychomotor Slowing:
   0  Normal speed of thinking, gesturing, and speaking.
   1  Patient notes slowed thinking, and voice modulation is reduced.
   2  Takes several seconds to respond to most questions; reports slowed thinking.
   3  Is largely unresponsive to most questions without strong encouragement.

16. Psychomotor Agitation:
   0  No increased speed or disorganization in thinking or gesturing.
   1  Fidgets, wrings hands and shifts positions often.
   2  Describes impulse to move about and displays motor restlessness.
   3  Unable to stay seated. Paces about with or without permission.

Enter the highest score on either of the 2 psychomotor items (15 or 16 above) __________

Total Score: _______(Range 0—27)
APPENDIX K

Consonant Vowel Consonant Task (CVC Task) (Baseline and Follow-up forms)
CVC Test: Recall Recording Sheet

<table>
<thead>
<tr>
<th>Date of Assessment</th>
<th>Participant Study Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer's Name</td>
<td>Mayouri Sukhapure</td>
</tr>
<tr>
<td>Interviewer's Number</td>
<td></td>
</tr>
</tbody>
</table>

Record CVCs in the order recalled. Include errors (CVCs not on list) and repetitions.

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Trial 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>List A</td>
<td>List A</td>
<td>List A</td>
<td>List A</td>
<td>List A</td>
<td>Delayed Recall</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>List A</td>
</tr>
</tbody>
</table>

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.
18.
19.
20.

Record all words that are said, even if they are incorrect. The score for each trial is the number of CVCs correctly recalled. Mark CVCs that are repeated with ‘R’, and errors (CVCs not on the list) with ‘E’.
CVC Verbal Learning Test: Recording Sheet for List A

<table>
<thead>
<tr>
<th>Date of Assessment</th>
<th>Participant Study Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer’s Name</td>
<td>Mayouri Sukhapure</td>
</tr>
<tr>
<td>Interviewer’s Number</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>List A</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Delayed Recall List A</th>
</tr>
</thead>
<tbody>
<tr>
<td>vev</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vob</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lec</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mub</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>jav</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>guz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Transfer each correct C-V-C from sheet 1. Record the C-V-C as a number depending on when it was recalled by the participant. Do not transfer repeats (R) or errors (E).
CVC Test: Recall Recording Sheet

<table>
<thead>
<tr>
<th>Date of Assessment</th>
<th>Participant Study Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer's Name</td>
<td>Mayouri Sukhapure</td>
</tr>
<tr>
<td>Interviewer's Number</td>
<td></td>
</tr>
</tbody>
</table>

Record CVCs in the order recalled. Include errors (CVCs not on list) and repetitions.

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Trial 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>List B</td>
<td>List B</td>
<td>List B</td>
<td>List B</td>
<td>List B</td>
<td>List B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Record all words that are said, even if they are incorrect. The score for each trial is the number of CVCs correctly recalled. Mark CVCs that are repeated with ‘R’, and errors (CVCs not on the list) with ‘E’.
CVC Verbal Learning Test: Recording Sheet List B

<table>
<thead>
<tr>
<th>Date of Assessment</th>
<th>Participant Study Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interviewer's Name</td>
<td>Mayouri Sukhapure</td>
</tr>
<tr>
<td>Interviewer's Number</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>List A</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
<th>Delayed Recall List B</th>
</tr>
</thead>
<tbody>
<tr>
<td>jiz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dav</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hif</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yoz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>jum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>caz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total**

Transfer each correct C-V-C from sheet 1. Record the C-V-C as a number depending on when it was recalled by the participant. Do not transfer repeats (R) or errors (E).
APPENDIX L

Visual Analogue Scale (VAS)
VISUAL ANALOGUE SCALE

ID: □ □ □ □ Date: …………………………… BASELINE / FOLLOW-UP

Circle one:

Time 0  Time 1  Time 2

Time: ………

1. Please rate the way you feel in terms of the dimension given below
2. Regard the line as representing the full range of the dimension
3. Rate your feelings as they are at the moment
4. Mark clearly and perpendicularly across each line

Example: ______________________________________

Not at all anxious ___________________________________ Worst anxiety ever
APPENDIX M

Trail Making Test (TMT) Part A and B
Trail Making Test (TMT) Parts A & B

Instructions:

Both parts of the Trail Making Test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 – 25, and the patient should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as in Part A, the patient draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The patient should be instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. Time the patient as he or she connects the “trail.” If the patient makes an error, point it out immediately and allow the patient to correct it. Errors affect the patient's score only in that the correction of errors is included in the completion time for the task. It is unnecessary to continue the test if the patient has not completed both parts after five minutes have elapsed.

Step 1: Give the patient a copy of the Trail Making Test Part A worksheet and a pen or pencil.
Step 2: Demonstrate the test to the patient using the sample sheet (Trail Making Part A – SAMPLE).
Step 3: Time the patient as he or she follows the “trail” made by the numbers on the test. Step 4: Record the time.
Step 5: Repeat the procedure for Trail Making Test Part B.

Scoring:

Results for both TMT A and B are reported as the number of seconds required to complete the task; therefore, higher scores reveal greater impairment.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Deficient</th>
<th>Rule of Thumb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trail A</td>
<td>29 seconds</td>
<td>&gt; 78 seconds</td>
<td>Most in 90 seconds</td>
</tr>
<tr>
<td>Trail B</td>
<td>75 seconds</td>
<td>&gt; 273 seconds</td>
<td>Most in 3 minutes</td>
</tr>
</tbody>
</table>

Sources:
- Gaudino EA, Geisler MW, Squires NK. Construct validity in the Trail Making Test: what makes

Patient’s Name: ____________________________  Date: ________________
Trail Making Test Part A – SAMPLE
Trail Making Test Part B

Patient's Name: ________________________________  Date: __________________

Diagram with numbered circles arranged randomly.
Trail Making Test Part B – SAMPLE
APPENDIX N

Digit Span Test (Baseline and Follow-up Tests)
Digit Span - Digits and Scoring Sheet - Baseline

**ID:**

Date of test: ....................................

DIGIT SPAN  Discontinue after failure on BOTH TRIALS of any item.
Administer BOTH TRIALS of each item, even if subject passes first trial

<table>
<thead>
<tr>
<th>Digits forward</th>
<th>Pass or Fail</th>
<th>Score 2, 1, or 0</th>
<th>Digits backward *</th>
<th>Pass or Fail</th>
<th>Score 2, 1, or 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 5 8 2</td>
<td></td>
<td></td>
<td>1 2 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 9 4</td>
<td></td>
<td></td>
<td>5 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 6 4 3 9</td>
<td></td>
<td>2 6 2 9</td>
<td>4 1 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 2 8 6</td>
<td></td>
<td>. 4 1 5</td>
<td>4 9 6 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 4 2 7 3 1</td>
<td></td>
<td>3 3 7 9</td>
<td>6 1 8 4 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 5 8 3 6</td>
<td></td>
<td>. 4 9 6 8</td>
<td>1 5 2 8 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 6 1 9 4 7 3</td>
<td></td>
<td>4 1 5 2 8 6</td>
<td>5 3 9 4 1 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 9 2 4 8 7</td>
<td></td>
<td>. 6 1 8 4 3</td>
<td>7 2 4 8 5 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 9 1 7 4 2 8</td>
<td></td>
<td>5 3 9 4 1 8</td>
<td>8 1 2 9 3 6 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 1 7 9 3 8 6</td>
<td></td>
<td>. 7 2 4 8 5 6</td>
<td>4 7 3 9 1 2 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 5 8 1 9 2 6 4</td>
<td></td>
<td>6 8 1 2 9 3 6 5</td>
<td>7 2 8 1 9 6 5 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 8 2 9 5 1 7 4</td>
<td></td>
<td>. 4 7 3 9 1 2 8</td>
<td>7 2 8 1 9 6 5 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total forward: Max = 14

Total backward: Max = 14

Grand total: Max = 28

Forward span: Max = 9

Backward span: Max = 8

*Administer Digits Backward even if subject scores 0 on Digits Forwards
**Digit Span - Digits and Scoring Sheet - 2nd testing session**

**ID:** [ ] [ ]

**Date of test:** ........................................

**DIGIT SPAN**

Discontinue after failure on BOTH TRIALS of any item.

Administer BOTH TRIALS of each item, even if subject passes first trial.

<table>
<thead>
<tr>
<th>Digits forward</th>
<th>Pass or Fail</th>
<th>Score 2, 1, or 0</th>
<th>Digits backward *</th>
<th>Pass or Fail</th>
<th>Score 2, 1, or 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 4 - 7 - 1</td>
<td></td>
<td>2</td>
<td>1 - 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - 8 - 3</td>
<td></td>
<td></td>
<td>4 - 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 5 - 3 - 2 - 8</td>
<td></td>
<td>2</td>
<td>5 - 1 - 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - 1 - 7 - 5</td>
<td></td>
<td></td>
<td>3 - 9 - 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 3 - 1 - 6 - 2 - 9</td>
<td></td>
<td>3</td>
<td>2 - 1 - 6 - 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - 4 - 7 - 2 - 5</td>
<td></td>
<td></td>
<td>3 - 8 - 5 - 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 5 - 9 - 8 - 3 - 6 - 2</td>
<td></td>
<td>4</td>
<td>9 - 4 - 1 - 7 - 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - 8 - 1 - 3 - 7 - 6</td>
<td></td>
<td></td>
<td>5 - 9 - 7 - 3 - 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 4 - 1 - 9 - 6 - 3 - 1 - 7</td>
<td></td>
<td>5</td>
<td>4 - 2 - 8 - 3 - 9 - 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - 9 - 6 - 1 - 2 - 7 - 5</td>
<td></td>
<td></td>
<td>6 - 1 - 3 - 7 - 4 - 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 4 - 7 - 1 - 9 - 3 - 5 - 2 - 6</td>
<td></td>
<td>6</td>
<td>7 - 9 - 1 - 8 - 2 - 5 - 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - 7 - 1 - 8 - 4 - 9 - 6 - 3</td>
<td></td>
<td></td>
<td>3 - 6 - 2 - 8 - 9 - 1 - 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 1 - 6 - 4 - 7 - 5 - 1 - 4 - 7 - 3</td>
<td></td>
<td>7</td>
<td>8 - 3 - 2 - 6 - 5 - 1 - 4 - 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - 9 - 2 - 8 - 3 - 1 - 4 - 5 - 7</td>
<td></td>
<td></td>
<td>6 - 1 - 7 - 9 - 8 - 5 - 4 - 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total forward:** Max = 14

**Total backward:** Max = 14

**Grand total:** Max = 28

**Forward span:** Max = 9

**Backward span:** Max = 8

*Administer DIGITS BACKWARD even if subject scores 0 on DIGITS FORWARDS*
APPENDIX O

Controlled Oral Word Association Test (COWAT): Baseline and Follow-up Measures
COWAT word recording sheet (Baseline)

Allow 90 seconds for each letter. If the participant discontinues before the end of the minute, encourage them to try and think of more words. If there is a silence of 15 seconds, repeat the basic instructions and the letter. Write down the words in the order in which they are produced. The score is the sum of all admissible words (exclude proper nouns, wrong words, variations, and repetitions). See normative data sheet for age and gender norms.

<table>
<thead>
<tr>
<th></th>
<th>Letter C</th>
<th>Letter F</th>
<th>Letter L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total:</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Grand total =
COWAT word recording sheet (Follow-up)

Allow 90 seconds for each letter. If the participant discontinues before the end of 90 seconds, encourage them to try and think of more words. If there is a silence of 15 seconds, repeat the basic instructions and the letter. Write down the words in the order in which they are produced. The score is the sum of all admissible words (exclude proper nouns, wrong words, variations, and repetitions).

<table>
<thead>
<tr>
<th></th>
<th>Letter P</th>
<th>Letter R</th>
<th>Letter W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Grand total = 
APPENDIX P

Reading the Mind in the Eyes Test (RMET)
practice

jealous

arrogant

panicked

hateful
playful

comforting

irritated

bored
terrified

upset

arrogant

annoyed
joking

flustered

desire

convinced
joking

insisting

amused

relaxed
irritated

sarcastic

worried

friendly
aghast

fantasizing

impatient

alarmed
apologetic  
friendly  

uneasy  

dispirited
despondent  relieved

shy  excited
annoyed

hostile

horrified

preoccupied
cautious      insisting

bored          aghast
terrified

amused

regretful

flirtatious
indifferent  embarrassed

sceptical  dispirited
decisive

anticipating

threatening

shy
irritated

disappointed

depressed

accusing
contemplative  flustered

encouraging  amused
irritated

thoughtful

encouraging

sympathetic
doubtful affectionate

playful aghast
decisive

amused

aghast

bored
arrogant          grateful
sarcastic          tentative
dominant, friendly, guilty, horrified
embarrassed

fantasizing

confused

panicked
preoccupied

grateful

insisting

imploring
contented  
apologetic

defiant  
curious
pensive

irritated

excited

hostile
panicked

derespondent

incredulous

interested
alarmed          shy

hostile         anxious
joking  cautious

arrogant  reassuring
interested

joking

affectionate

contented
impatient

aghast

irritated

reflective
grateful
flirtatious

hostile
disappointed
ashamed  confident

joking  dispirited
serious ashamed

bewildered alarmed
embarrassed

fantasizing

concerned

guilty
aghast

baffled

distrustful
terrified
puzzled
nervous

insisting
contemplative
ashamed

nervous

suspicious

indecisive
For all users of the revised version of the Adult “Reading the Mind in the Eyes” Test.

Enclosed you will find

- the adult version of the above test
- the word definition handout,
- the correct answers.
- A copy of the paper describing the test in full

As you know, publication details of the original version appeared in the *Journal of Child Psychology and Psychiatry*, 38, 813-822 (1997). The revised version which we have sent you was published in the *Journal of Child Psychiatry and Psychiatry*, 42, 241-252 (2001).

A child version of this test has also been developed and is available upon request. It was published in the *Journal of Developmental and Learning Disorders*, 5, 47-78 (2001).

We would, of course, appreciate hearing of any results you obtain with this test.

Thank you.

Best wishes

Simon Baron-Cohen
Adult Eyes Instructions

For each set of eyes, choose and circle which word best describes what the person in the picture is thinking or feeling. You may feel that more than one word is applicable but please choose just one word, the word which you consider to be most suitable. Before making your choice, make sure that you have read all 4 words. You should try to do the task as quickly as possible but you will not be timed. If you really don't know what a word means you can look it up in the definition handout.
**WORD DEFINITIONS**

**ACCUSING**
blaming
The policeman was accusing the man of stealing a wallet.

**AFFECTIONATE**
showing fondness towards someone
Most mothers are affectionate to their babies by giving them lots of kisses and cuddles.

**AGHAST**
horrified, astonished, alarmed
Jane was aghast when she discovered her house had been burgled.

**ALARMED**
fearful, worried, filled with anxiety
Claire was alarmed when she thought she was being followed home.

**AMUSED**
finding something funny
I was amused by a funny joke someone told me.

**ANNOYED**
irritated, displeased
Jack was annoyed when he found out he had missed the last bus home.

**ANTICIPATING**
expecting
At the start of the football match, the fans were anticipating a quick goal.

**ANXIOUS**
worried, tense, uneasy
The student was feeling anxious before taking her final exams.

**APOLOGETIC**
feeling sorry
The waiter was very apologetic when he spilt soup all over the customer.

**ARROGANT**
conceited, self-important, having a big opinion of oneself
The arrogant man thought he knew more about politics than everyone else in the room.

**ASHAMED**
overcome with shame or guilt
The boy felt ashamed when his mother discovered him stealing money from her purse.
<table>
<thead>
<tr>
<th>ASSERTIVE</th>
<th>confident, dominant, sure of oneself</th>
</tr>
</thead>
<tbody>
<tr>
<td>The assertive woman demanded that the shop give her a refund.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BAFFLED</th>
<th>confused, puzzled, dumbfounded</th>
</tr>
</thead>
<tbody>
<tr>
<td>The detectives were completely baffled by the murder case.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BEWILDERED</th>
<th>utterly confused, puzzled, dazed</th>
</tr>
</thead>
<tbody>
<tr>
<td>The child was bewildered when visiting the big city for the first time.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CAUTIOUS</th>
<th>careful, wary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarah was always a bit cautious when talking to someone she did not know.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>COMFORTING</th>
<th>consoling, compassionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>The nurse was comforting the wounded soldier.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CONCERNED</th>
<th>worried, troubled</th>
</tr>
</thead>
<tbody>
<tr>
<td>The doctor was concerned when his patient took a turn for the worse.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CONFIDENT</th>
<th>self-assured, believing in oneself</th>
</tr>
</thead>
<tbody>
<tr>
<td>The tennis player was feeling very confident about winning his match.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CONFUSED</th>
<th>puzzled, perplexed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lizzie was so confused by the directions given to her, she got lost.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CONTEMPLATIVE</th>
<th>reflective, thoughtful, considering</th>
</tr>
</thead>
<tbody>
<tr>
<td>John was in a contemplative mood on the eve of his 60th birthday.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CONTENTED</th>
<th>satisfied</th>
</tr>
</thead>
<tbody>
<tr>
<td>After a nice walk and a good meal, David felt very contented.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CONVINCED</th>
<th>certain, absolutely positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richard was convinced he had come to the right decision.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CURIOUS</th>
<th>inquisitive, inquiring, prying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louise was curious about the strange shaped parcel.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DECIDING</th>
<th>making your mind up</th>
</tr>
</thead>
<tbody>
<tr>
<td>The man was deciding whom to vote for in the election.</td>
<td></td>
</tr>
</tbody>
</table>
DECISIVE already made your mind up
Jane looked very decisive as she walked into the polling station.

DEFIANT insolent, bold, don't care what anyone else thinks
The animal protester remained defiant even after being sent to prison.

DEPRESSED miserable
George was depressed when he didn't receive any birthday cards.

DESIRE passion, lust, longing for
Kate had a strong desire for chocolate.

DESPIRITED glum, miserable, low
Adam was dispirited when he failed his exams.

DISTRUSTFUL suspicious, doubtful, wary
The old woman was distrustful of the stranger at her door.

DOMINANT commanding, bossy
The sergeant major looked dominant as he inspected the new recruits.

DOUBTFUL dubious, suspicious, not really believing
Mary was doubtful that her son was telling the truth.

DUBIOUS doubtful, suspicious
Peter was dubious when offered a surprisingly cheap television in a pub.

EAGER keen
On Christmas morning, the children were eager to open their presents.

EARNEST having a serious intention
Harry was very earnest about his religious beliefs.
EMBARRASSED  ashamed
After forgetting a colleague's name, Jenny felt very embarrassed.

ENCOURAGING  hopeful, heartening, supporting
All the parents were encouraging their children in the school sports day.

ENTERTAINED  absorbed and amused or pleased by something
I was very entertained by the magician.

ENTHUSIASTIC  very eager, keen
Susan felt very enthusiastic about her new fitness plan.

FANTASIZING  daydreaming
Emma was fantasizing about being a film star.

FASCINATED  captivated, really interested
At the seaside, the children were fascinated by the creatures in the rock pools.

FEARFUL  terrified, worried
In the dark streets, the women felt fearful.

FLIRTATIOUS  brazen, saucy, teasing, playful
Connie was accused of being flirtatious when she winked at a stranger at a party.

FLUSTERED  confused, nervous and upset
Sarah felt a bit flustered when she realised how late she was for the meeting and that she had forgotten an important document.

FRIENDLY  sociable, amiable
The friendly girl showed the tourists the way to the town centre.

GRATEFUL  thankful
Kelly was very grateful for the kindness shown by the stranger.

GUILTY  feeling sorry for doing something wrong
Charlie felt guilty about having an affair.

HATEFUL  showing intense dislike
The two sisters were hateful to each other and always fighting.
HOPEFUL  optimistic
Larry was hopeful that the post would bring good news.

HORRIFIED  terrified, appalled
The man was horrified to discover that his new wife was already married.

HOSTILE  unfriendly
The two neighbours were hostile towards each other because of an argument about loud music.

IMPATIENT  restless, wanting something to happen soon
Jane grew increasingly impatient as she waited for her friend who was already 20 minutes late.

IMPLORING  begging, pleading
Nicola looked imploring as she tried to persuade her dad to lend her the car.

INCREDOUS  not believing
Simon was incredulous when he heard that he had won the lottery.

INDECISIVE  unsure, hesitant, unable to make your mind up
Tammy was so indecisive that she couldn't even decide what to have for lunch.

INDIFFERENT  disinterested, unresponsive, don't care
Terry was completely indifferent as to whether they went to the cinema or the pub.

INSISTING  demanding, persisting, maintaining
After a work outing, Frank was insisting he paid the bill for everyone.

INSULTING  rude, offensive
The football crowd was insulting the referee after he gave a penalty.

INTERESTED  inquiring, curious
After seeing Jurassic Park, Hugh grew very interested in dinosaurs.

INTRIGUED  very curious, very interested
A mystery phone call intrigued Zoe.
IRRITATED  exasperated, annoyed
Frances was irritated by all the junk mail she received.

JEALOUS  envious
Tony was jealous of all the taller, better-looking boys in his class.

JOKING  being funny, playful
Gary was always joking with his friends.

NERVOUS  apprehensive, tense, worried
Just before her job interview, Alice felt very nervous.

OFFENDED  insulted, wounded, having hurt feelings
When someone made a joke about her weight, Martha felt very offended.

PANICKED  distraught, feeling of terror or anxiety
On waking to find the house on fire, the whole family was panicked.

PENSIVE  thinking about something slightly worrying
Susie looked pensive on the way to meeting her boyfriend's parents for the first time.

PERPLEXED  bewildered, puzzled, confused
Frank was perplexed by the disappearance of his garden gnomes.

PLAYFUL  full of high spirits and fun
Neil was feeling playful at his birthday party.

PREOCCUPIED  absorbed, engrossed in one's own thoughts
Worrying about her mother's illness made Debbie preoccupied at work.

PUZZLED  perplexed, bewildered, confused
After doing the crossword for an hour, June was still puzzled by one clue.

REASSURING  supporting, encouraging, giving someone confidence
Andy tried to look reassuring as he told his wife that her new dress did suit her.

REFLECTIVE  contemplative, thoughtful
George was in a reflective mood as he thought about what he'd done with his life.

REGRETFUL  sorry
Lee was always regretful that he had never travelled when he was younger.

**RELAXED**
- taking it easy, calm, carefree

On holiday, Pam felt happy and relaxed.

**RELIEVED**
- freed from worry or anxiety

At the restaurant, Ray was relieved to find that he had not forgotten his wallet.

**RESENTFUL**
- bitter, hostile

The businessman felt very resentful towards his younger colleague who had been promoted above him.

**SARCASTIC**
- cynical, mocking, scornful

The comedian made a sarcastic comment when someone came into the theatre late.

**SATISFIED**
- content, fulfilled

Steve felt very satisfied after he had got his new flat just how he wanted it.

**SCEPTICAL**
- doubtful, suspicious, mistrusting

Patrick looked sceptical as someone read out his horoscope to him.

**SERIOUS**
- solemn, grave

The bank manager looked serious as he refused Nigel an overdraft.

**STERN**
- severe, strict, firm

The teacher looked very stern as he told the class off.

**SUSPICIOUS**
- disbeliefing, suspecting, doubting

After Sam had lost his wallet for the second time at work, he grew suspicious of one of his colleagues.

**SYMPATHETIC**
- kind, compassionate

The nurse looked sympathetic as she told the patient the bad news.

**TENTATIVE**
- hesitant, uncertain, cautious

Andrew felt a bit tentative as he went into the room full of strangers.

**TERRIFIED**
- alarmed, fearful

The boy was terrified when he thought he saw a ghost.

**THOUGHTFUL**
- thinking about something
Phil looked thoughtful as he sat waiting for the girlfriend he was about to finish with.

**THREATENING**
- menacing, intimidating
  - The large, drunken man was acting in a very threatening way.

**UNEASY**
- unsettled, apprehensive, troubled
  - Karen felt slightly uneasy about accepting a lift from the man she had only met that day.

**UPSET**
- agitated, worried, uneasy
  - The man was very upset when his mother died.

**WORRIED**
- anxious, fretful, troubled
  - When her cat went missing, the girl was very worried.
# Record Sheet

**Date of Birth:**

**Today’s date:**

**Degree subject/occupation:**

<table>
<thead>
<tr>
<th></th>
<th>jealous</th>
<th>panicked</th>
<th>arrogant</th>
<th>hateful</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>playful</td>
<td>comforting</td>
<td>irritated</td>
<td>bored</td>
</tr>
<tr>
<td>2</td>
<td>terrified</td>
<td>upset</td>
<td>arrogant</td>
<td>annoyed</td>
</tr>
<tr>
<td>3</td>
<td>joking</td>
<td>flustered</td>
<td>desire</td>
<td>convinced</td>
</tr>
<tr>
<td>4</td>
<td>joking</td>
<td>insisting</td>
<td>amused</td>
<td>relaxed</td>
</tr>
<tr>
<td>5</td>
<td>irritated</td>
<td>sarcastic</td>
<td>worried</td>
<td>friendly</td>
</tr>
<tr>
<td>6</td>
<td>aghast</td>
<td>fantasizing</td>
<td>impatient</td>
<td>alarmed</td>
</tr>
<tr>
<td>7</td>
<td>apologetic</td>
<td>friendly</td>
<td>uneasy</td>
<td>dispirited</td>
</tr>
<tr>
<td>8</td>
<td>despondent</td>
<td>relieved</td>
<td>shy</td>
<td>excited</td>
</tr>
<tr>
<td>9</td>
<td>annoyed</td>
<td>hostile</td>
<td>horrified</td>
<td>preoccupied</td>
</tr>
<tr>
<td>10</td>
<td>cautious</td>
<td>insisting</td>
<td>bored</td>
<td>aghast</td>
</tr>
<tr>
<td>11</td>
<td>terrified</td>
<td>amused</td>
<td>regretful</td>
<td>flirtatious</td>
</tr>
<tr>
<td>12</td>
<td>indifferent</td>
<td>embarrassed</td>
<td>sceptical</td>
<td>dispirited</td>
</tr>
<tr>
<td>13</td>
<td>decisive</td>
<td>anticipating</td>
<td>threatening</td>
<td>shy</td>
</tr>
<tr>
<td>14</td>
<td>irritated</td>
<td>disappointed</td>
<td>depressed</td>
<td>accusing</td>
</tr>
<tr>
<td>15</td>
<td>contemplative</td>
<td>flustered</td>
<td>encouraging</td>
<td>amused</td>
</tr>
<tr>
<td>16</td>
<td>irritated</td>
<td>thoughtful</td>
<td>encouraging</td>
<td>sympathetic</td>
</tr>
<tr>
<td>17</td>
<td>doubtful</td>
<td>affectionate</td>
<td>playful</td>
<td>aghast</td>
</tr>
<tr>
<td>18</td>
<td>decisive</td>
<td>amused</td>
<td>aghast</td>
<td>bored</td>
</tr>
<tr>
<td>19</td>
<td>arrogant</td>
<td>grateful</td>
<td>sarcastic</td>
<td>tentative</td>
</tr>
<tr>
<td>20</td>
<td>dominant</td>
<td>friendly</td>
<td>guilty</td>
<td>horrified</td>
</tr>
<tr>
<td>21</td>
<td>embarrassed</td>
<td>fantasizing</td>
<td>confused</td>
<td>panicked</td>
</tr>
<tr>
<td>22</td>
<td>preoccupied</td>
<td>grateful</td>
<td>insisting</td>
<td>imploring</td>
</tr>
<tr>
<td>23</td>
<td>contented</td>
<td>apologetic</td>
<td>defiant</td>
<td>curious</td>
</tr>
<tr>
<td>24</td>
<td>pensive</td>
<td>irritated</td>
<td>excited</td>
<td>hostile</td>
</tr>
<tr>
<td>25</td>
<td>panicked</td>
<td>incredulous</td>
<td>despondent</td>
<td>interested</td>
</tr>
<tr>
<td>26</td>
<td>alarmed</td>
<td>shy</td>
<td>hostile</td>
<td>anxious</td>
</tr>
<tr>
<td>27</td>
<td>joking</td>
<td>cautious</td>
<td>arrogant</td>
<td>reassuring</td>
</tr>
<tr>
<td>28</td>
<td>interested</td>
<td>joking</td>
<td>affectionate</td>
<td>contented</td>
</tr>
<tr>
<td>29</td>
<td>impatient</td>
<td>aghast</td>
<td>irritated</td>
<td>reflective</td>
</tr>
<tr>
<td>30</td>
<td>grateful</td>
<td>flirtatious</td>
<td>hostile</td>
<td>disappointed</td>
</tr>
<tr>
<td>31</td>
<td>ashamed</td>
<td>confident</td>
<td>joking</td>
<td>dispirited</td>
</tr>
<tr>
<td>32</td>
<td>serious</td>
<td>ashamed</td>
<td>bewilderded</td>
<td>alarmed</td>
</tr>
<tr>
<td>33</td>
<td>embarrassed</td>
<td>guilty</td>
<td>fantasizing</td>
<td>concerned</td>
</tr>
<tr>
<td>34</td>
<td>aghast</td>
<td>baffled</td>
<td>distrustful</td>
<td>terrified</td>
</tr>
<tr>
<td>35</td>
<td>puzzled</td>
<td>nervous</td>
<td>insisting</td>
<td>contemplative</td>
</tr>
<tr>
<td>36</td>
<td>ashamed</td>
<td>nervous</td>
<td>suspicious</td>
<td>indecisive</td>
</tr>
<tr>
<td>Answers - Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. jealous</td>
<td>panicked</td>
<td>arrogant</td>
<td>hateful</td>
<td>M</td>
</tr>
<tr>
<td>2. playf</td>
<td>comforting</td>
<td>irritated</td>
<td>bored</td>
<td>M</td>
</tr>
<tr>
<td>3. terrified</td>
<td>upset</td>
<td>arrogant</td>
<td>annoyed</td>
<td>M</td>
</tr>
<tr>
<td>4. joking</td>
<td>desire</td>
<td>convinced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. joking</td>
<td>insisting</td>
<td>amused</td>
<td>relaxed</td>
<td>M</td>
</tr>
<tr>
<td>6. irritated</td>
<td>sarcastic</td>
<td>worried</td>
<td>friendly</td>
<td>M</td>
</tr>
<tr>
<td>7. aghast</td>
<td>fantasizing</td>
<td>impatient</td>
<td>alarmed</td>
<td>F</td>
</tr>
<tr>
<td>8. apologetic</td>
<td>friendly</td>
<td>uneasy</td>
<td>dispirited</td>
<td>M</td>
</tr>
<tr>
<td>9. despondent</td>
<td>relieved</td>
<td>shy</td>
<td>excited</td>
<td>M</td>
</tr>
<tr>
<td>10. annoyed</td>
<td>hostile</td>
<td>horrified</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>11. cautious</td>
<td>insisting</td>
<td>bored</td>
<td>aghast</td>
<td>M</td>
</tr>
<tr>
<td>12.ersonic</td>
<td>opposing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. dominated</td>
<td>friendly</td>
<td>guilty</td>
<td>horrified</td>
<td>M</td>
</tr>
<tr>
<td>14. embarrassed</td>
<td>fantasizing</td>
<td>confused</td>
<td>panicked</td>
<td>F</td>
</tr>
<tr>
<td>15. preoccupied</td>
<td>grateful</td>
<td>insisting</td>
<td>imploring</td>
<td>F</td>
</tr>
<tr>
<td>16. contented</td>
<td>apologetic</td>
<td>defiant</td>
<td>curious</td>
<td>M</td>
</tr>
<tr>
<td>17. pensive</td>
<td>irritated</td>
<td>excited</td>
<td>hostile</td>
<td>M</td>
</tr>
<tr>
<td>18. panicked</td>
<td>incredulous</td>
<td>interested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. alarmed</td>
<td>shy</td>
<td>hostile</td>
<td>anxious</td>
<td>M</td>
</tr>
<tr>
<td>20. joking</td>
<td>cautious</td>
<td>arrogant</td>
<td>reassuring</td>
<td>F</td>
</tr>
<tr>
<td>21. interested</td>
<td>joking</td>
<td>affectionate</td>
<td>contented</td>
<td>F</td>
</tr>
<tr>
<td>22. impatient</td>
<td>aghast</td>
<td>irritated</td>
<td>reflective</td>
<td>F</td>
</tr>
<tr>
<td>23. grateful</td>
<td>flirtatious</td>
<td>hostile</td>
<td>disappointed</td>
<td>F</td>
</tr>
<tr>
<td>24. ashamed</td>
<td>confident</td>
<td>joking</td>
<td>disappointed</td>
<td>F</td>
</tr>
<tr>
<td>25. serious</td>
<td>ashamed</td>
<td>bewildered</td>
<td>alarmed</td>
<td>M</td>
</tr>
<tr>
<td>26. embarrassed</td>
<td>guilty</td>
<td>anticsizing</td>
<td>concerned</td>
<td>M</td>
</tr>
<tr>
<td>27. aghast</td>
<td>baffled</td>
<td>distrustful</td>
<td>terrified</td>
<td>F</td>
</tr>
<tr>
<td>28. puzzled</td>
<td>nervous</td>
<td>insisting</td>
<td>contemplative</td>
<td>F</td>
</tr>
<tr>
<td>29. ashamed</td>
<td>nervous</td>
<td>suspicious</td>
<td>indecisive</td>
<td>M</td>
</tr>
</tbody>
</table>
APPENDIX Q

Research Request Form
## TESTS REQUIRED:

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TEST</strong></td>
<td>Biochemistry Steroid Lab 1 x Li Hep</td>
</tr>
<tr>
<td><strong>SHBG</strong></td>
<td>Biochemistry Steroid Lab share 1 x Li Hep</td>
</tr>
<tr>
<td><strong>LH</strong></td>
<td>Endocrine Laboratory – 1 x 4.5mL Lith Hep (share with Steroid lab)</td>
</tr>
<tr>
<td><strong>FSH</strong></td>
<td>Endocrine Laboratory – 1 x 4.5mL Lith Hep (share with Steroid lab)</td>
</tr>
</tbody>
</table>

Specimen requirements 1 x Lith Hep 4.5mL.
APPENDIX R

Order of Cognitive Tests Included in the Current Study
SESSION #1 – PATIENT

Date ............................................................... MALE / FEMALE

Subject .......................................................... Subject Number ......................

Address: ........................................................................................................................................

Phone: ............................................. or ....................................................

E-mail: .................................................................................................

TESTS TO RUN

☐ Demo Ques
☐ NART
☐ VAS Time 0
☐ CVC- Immediate Trial
☐ Trail Making A & B
☐ FER
☐ CVC Delayed Trial+ Recognition
☐ VAS Time 1
☐ Timed Chase Test- CogState
☐ GMLT immediate trial -CogState
☐ COWAT
☐ GMLT delay trial- CogState
☐ Digit Span
☐ R-MET
☐ VAS Time 2
APPENDIX S

Tables Showing Results Showing Associations between Testosterone Levels, Symptoms of Depression and Anxiety and Cognitive Function: Raw and Adjusted Verbal IQ (NART) Scores
Table 1

*Mean Scores (SD), Adjusted Means (SEM), and Effect Sizes on the Consonant-Vowel-Consonant Test in the Polycystic Ovarian Syndrome (n=50) and Control (n=53) Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS Mean</th>
<th>SD / SEM</th>
<th>Controls Mean</th>
<th>SD / SEM</th>
<th>F</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CVC Trial 1</strong></td>
<td>Raw</td>
<td>3.62</td>
<td>1.53</td>
<td>3.66</td>
<td>1.77</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>3.70</td>
<td>0.24</td>
<td>3.57</td>
<td>0.23</td>
<td>2.32</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>CVC Trial 5</strong></td>
<td>Raw</td>
<td>8.08</td>
<td>3.39</td>
<td>8.91</td>
<td>2.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>8.26</td>
<td>0.45</td>
<td>8.73</td>
<td>0.44</td>
<td>2.26</td>
<td>0.136</td>
</tr>
<tr>
<td><strong>CVC Total</strong></td>
<td>Raw</td>
<td>30.58</td>
<td>11.62</td>
<td>33.08</td>
<td>11.47</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>31.51</td>
<td>1.64</td>
<td>32.19</td>
<td>1.59</td>
<td>5.74</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Delayed Recall</strong></td>
<td>Raw</td>
<td>6.50</td>
<td>3.33</td>
<td>7.00</td>
<td>3.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>6.68</td>
<td>0.48</td>
<td>6.82</td>
<td>0.47</td>
<td>2.62</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Recognition</strong></td>
<td>Raw</td>
<td>13.94</td>
<td>1.39</td>
<td>14.30</td>
<td>0.84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>13.90</td>
<td>0.16</td>
<td>14.34</td>
<td>0.16</td>
<td>0.99</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Raw and adjusted means are presented, with the latter using NART as a covariate; $F$ = univariate ANCOVA, $d$ = Cohen’s $d$ effect size, CVC = Consonant-Vowel-Consonant, PCOS = Polycystic Ovarian Syndrome.
Table 2

Mean Total Errors (SD and SEM) and Effect Sizes for the Groton Maze Learning Test in the Polycystic Ovarian Syndrome (n=50) and Control (n=53) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Controls</th>
<th>F</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD/SEM</td>
<td>Mean</td>
<td>SD/SEM</td>
<td></td>
</tr>
<tr>
<td>GMLT Total</td>
<td>Raw 15.36</td>
<td>2.71</td>
<td>15.77</td>
<td>3.02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 15.41</td>
<td>0.42</td>
<td>15.73</td>
<td>0.41</td>
<td>0.24</td>
</tr>
<tr>
<td>GMLT Trial 1</td>
<td>Raw 5.86</td>
<td>3.92</td>
<td>4.57</td>
<td>2.99</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 5.69</td>
<td>0.50</td>
<td>4.73</td>
<td>0.49</td>
<td>1.99</td>
</tr>
<tr>
<td>GMLT Trial 5</td>
<td>Raw 45.60</td>
<td>12.32</td>
<td>42.04</td>
<td>11.09</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 44.90</td>
<td>1.69</td>
<td>42.70</td>
<td>1.64</td>
<td>3.00</td>
</tr>
<tr>
<td>GMLT Trials 1-5</td>
<td>Raw 5.22</td>
<td>3.59</td>
<td>4.47</td>
<td>2.97</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 5.12</td>
<td>0.48</td>
<td>4.56</td>
<td>0.46</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Adjusted means after covarying only for NART. F= univariate ANCOVA, d = Cohen’s d effect size, GMLT- Groton Maze Learning Test, PCOS- Polycystic Ovarian Syndrome.
Table 3

*Means (SD) and Effect Sizes for Psychomotor Speed Measures in the Polycystic Ovarian Syndrome (n=50) and Control (n=53) Groups*

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>F</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Timed Chase Test (no of correct moves)</td>
<td>Raw 43.42</td>
<td>7.12</td>
<td>49.13</td>
<td>7.99</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 43.37</td>
<td>1.11</td>
<td>49.18</td>
<td>1.07</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Raw</td>
<td>-</td>
<td>0.60</td>
<td>1.14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>0.62</td>
<td>0.18</td>
<td>0.66</td>
<td>0.17</td>
</tr>
<tr>
<td>Trail Making Errors List A</td>
<td>Raw 0.58</td>
<td>0.73</td>
<td>0.53</td>
<td>0.82</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>0.56</td>
<td>0.11</td>
<td>0.55</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Adjusted means using NART as a covariate, \( F = \) univariate ANCOVA, \( d = \) Cohen’s d effect size, **PCOS**- Polycystic Ovarian Syndrome
Table 4

Means (SD and SEM) and Effect Sizes on the Trail Making Test (List B), COWAT, and the Digit Span Task in the Polycystic Ovarian Syndrome (n=50) and Control (n=53) Groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Controls</th>
<th>( F^a )</th>
<th>( p^a )</th>
<th>( d^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trail Making time List B (seconds)</strong></td>
<td>Raw 46.52 18.18 42.17 18.18</td>
<td>Raw 42.17 18.18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 44.48 2.52 44.09 2.44</td>
<td>Adjusted 44.09 2.44</td>
<td>11.54</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Trail Making errors List B</strong></td>
<td>Raw 1.22 2.03 1.34 1.79</td>
<td>Raw 1.34 1.79</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 1.24 0.28 1.32 0.27</td>
<td>Adjusted 1.32 0.27</td>
<td>0.07</td>
<td>0.784</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>COWAT Total Score</strong></td>
<td>Raw 47.60 14.10 56.42 15.15</td>
<td>Raw 56.42 15.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 49.53 1.98 54.59 1.92</td>
<td>Adjusted 54.59 1.92</td>
<td>16.65</td>
<td>&lt;0.001</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Digit Span Total Forward</strong></td>
<td>Raw 8.40 2.50 9.42 2.26</td>
<td>Raw 9.42 2.26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 8.79 0.31 9.04 0.30</td>
<td>Adjusted 9.04 0.30</td>
<td>28.84</td>
<td>&lt;0.001</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Digit Span Forward Span</strong></td>
<td>Raw 6.74 1.32 7.25 1.31</td>
<td>Raw 7.25 1.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 6.94 0.17 7.06 0.17</td>
<td>Adjusted 7.06 0.17</td>
<td>22.24</td>
<td>&lt;0.001</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Digit Span Total Backward</strong></td>
<td>Raw 6.54 2.04 7.62 2.46</td>
<td>Raw 7.62 2.46</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 6.87 0.30 7.31 0.29</td>
<td>Adjusted 7.31 0.29</td>
<td>21.56</td>
<td>&lt;0.001</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Digit Span Backward Span</strong></td>
<td>Raw 4.82 1.19 5.30 1.32</td>
<td>Raw 5.30 1.32</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 5.00 0.17 5.13 0.16</td>
<td>Adjusted 5.13 0.16</td>
<td>21.26</td>
<td>&lt;0.001</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Digit Span Total Score</strong></td>
<td>Raw 14.94 3.88 17.04 4.30</td>
<td>Raw 17.04 4.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted 15.67 0.52 16.35 0.50</td>
<td>Adjusted 16.35 0.50</td>
<td>34.55</td>
<td>&lt;0.001</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Adjusted means after covarying for NART, \( F \)=univariate ANCOVA, \( d \)=Cohen’s d effect size, COWAT=Controlled Oral Word Association Test, PCOS=Polycystic Ovarian Syndrome
Table 5

Correlations between Testosterone Variables and Depression and Anxiety Variables scores across the Entire Sample (n=103)

<table>
<thead>
<tr>
<th></th>
<th>HADS-D</th>
<th>HADS-A</th>
<th>HADS total score</th>
<th>QIDS Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>0.007</td>
<td>-0.01</td>
<td>-0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>-0.01</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.01</td>
</tr>
<tr>
<td><strong>Free T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>0.33**</td>
<td>0.09</td>
<td><strong>0.23</strong></td>
<td><strong>0.24</strong></td>
</tr>
<tr>
<td>Adjusted-</td>
<td>0.28</td>
<td>0.04</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>0.37**</td>
<td>0.11</td>
<td><strong>0.27</strong></td>
<td><strong>0.31</strong></td>
</tr>
<tr>
<td>Adjusted-</td>
<td><strong>0.32</strong></td>
<td>0.05</td>
<td><strong>0.21</strong></td>
<td><strong>0.26</strong></td>
</tr>
<tr>
<td><strong>SHBG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.28</td>
<td>-0.03</td>
<td>-0.17</td>
<td>-0.17</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>-0.25**</td>
<td>-0.00</td>
<td>-0.14</td>
<td>-0.14</td>
</tr>
</tbody>
</table>

HADS= Hospital Anxiety and Depression Scale, HADS-A= Hospital Anxiety and Depression Scale (Anxiety sub-scale), HADS-D= Hospital Anxiety and Depression Scale (Depression sub-scale), QIDS= Quick inventory of Depressive Symptomatology, FAI= Free Androgen Index, Free T= Free Testosterone, Total T= Total Testosterone, *=<0.05, ** =<0.01
Table 6

*Correlations between Testosterone Variables and Consonant Vowel Consonant Task (CVC) Variables across the Entire Sample (n=103)*

<table>
<thead>
<tr>
<th></th>
<th>CVC Trial 1</th>
<th>CVC Trial 5</th>
<th>CVC Total Learning</th>
<th>CVC Delayed Recall</th>
<th>CVC Recognition accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.007</td>
<td>-0.078</td>
<td>-0.036</td>
<td>-0.025</td>
<td>-0.187</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.002</td>
<td>-0.068</td>
<td>-0.022</td>
<td>-0.015</td>
<td>-0.190</td>
</tr>
<tr>
<td><strong>Free T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.066</td>
<td>-0.110</td>
<td>-0.085</td>
<td>-0.042</td>
<td><strong>-0.221</strong>*</td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.038</td>
<td>-0.075</td>
<td>-0.038</td>
<td>-0.008</td>
<td><strong>-0.233</strong>*</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.089</td>
<td>-0.114</td>
<td>-0.100</td>
<td>-0.034</td>
<td>-0.192</td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.060</td>
<td>-0.076</td>
<td>-0.048</td>
<td>0.004</td>
<td><strong>-0.206</strong>*</td>
</tr>
</tbody>
</table>

CVC- Consonant Vowel Consonant Task, FAI- Free Androgen Index, Free T- Free Testosterone, Total T- Total Testosterone, *=<0.05, **=<0.01
Table 7

Correlations between Testosterone Variables and Groton Maze Learning Test Variables across the Entire Sample (n=103)

<table>
<thead>
<tr>
<th></th>
<th>GMLT Total Errors Trial 1</th>
<th>GMLT Total Errors Trial 5</th>
<th>GMLT Total Errors Trials 1-5</th>
<th>GMLT Delay Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.012</td>
<td>0.168</td>
<td>0.177</td>
<td>0.024</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>-0.008</td>
<td>0.160</td>
<td>0.107</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Free</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>0.136</td>
<td><strong>0.274</strong></td>
<td><strong>0.259</strong></td>
<td>0.093</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>0.153</td>
<td><strong>0.246</strong></td>
<td><strong>0.228</strong></td>
<td>0.073</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>0.144</td>
<td>0.202</td>
<td><strong>0.204</strong></td>
<td>0.035</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>0.163</td>
<td>0.168</td>
<td>0.167</td>
<td>0.011</td>
</tr>
</tbody>
</table>

GMLT- Groton Maze Learning Test, FAI- Free Androgen Index, Free T- Free Testosterone, Total T- Total Testosterone, *=<0.05, **=<0.01

Table 8

Correlations between Testosterone Variables and Psychomotor Speed Variables across the Entire Sample (n=103)

<table>
<thead>
<tr>
<th></th>
<th>TCT (No of Correct Moves)</th>
<th>TCT (Total Errors)</th>
<th>TMT List A (Time)</th>
<th>TMT List A (Errors)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.141</td>
<td>0.093</td>
<td>0.123</td>
<td>0.031</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>-0.136</td>
<td>0.096</td>
<td>0.108</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>Free T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.321*</td>
<td>&lt;0.001</td>
<td>-0.044</td>
<td>-0.086</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>-0.310*</td>
<td>0.010</td>
<td>-0.143</td>
<td>-0.106</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw-</td>
<td>-0.276**</td>
<td>-0.020</td>
<td>-0.050</td>
<td>-0.103</td>
</tr>
<tr>
<td>Adjusted-</td>
<td>-0.262**</td>
<td>-0.010</td>
<td>-0.159</td>
<td>-0.125</td>
</tr>
</tbody>
</table>

TCT- Timed Chase Test, TMT- Trail Making Test (Part A), FAI- Free Androgen Index, Free T- Free Testosterone, Total T- Total Testosterone, *=<0.05, **=<0.01
<table>
<thead>
<tr>
<th></th>
<th>TMT Part B (Time)</th>
<th>TMT Part B (Errors)</th>
<th>COWAT Total Score</th>
<th>Digit Span Total Forward</th>
<th>Digit Span Total Span</th>
<th>Digit Span Total Backward Span</th>
<th>Digit Span Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total T</strong></td>
<td>Raw- 0.200*</td>
<td>0.056</td>
<td>-0.150</td>
<td>-0.024</td>
<td>0.006</td>
<td>0.001</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Adjusted- 0.192</td>
<td>0.058</td>
<td>-0.139</td>
<td>0.006</td>
<td>0.037</td>
<td>0.032</td>
<td>0.039</td>
</tr>
<tr>
<td><strong>Free T</strong></td>
<td>Raw- 0.160</td>
<td>-0.019</td>
<td>-0.231*</td>
<td>-0.223*</td>
<td>-0.246*</td>
<td>-0.057</td>
<td>-0.079</td>
</tr>
<tr>
<td></td>
<td>Adjusted- 0.102</td>
<td>-0.012</td>
<td>-0.165</td>
<td>-0.148</td>
<td>-0.180</td>
<td>0.038</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>FAI</strong></td>
<td>Raw- 0.122</td>
<td>-0.048</td>
<td>-0.195</td>
<td>-0.194</td>
<td>-0.199</td>
<td>-0.071</td>
<td>-0.101</td>
</tr>
<tr>
<td></td>
<td>Adjusted- 0.053</td>
<td>-0.042</td>
<td>-0.116</td>
<td>-0.102</td>
<td>-0.117</td>
<td>0.033</td>
<td>-0.005</td>
</tr>
</tbody>
</table>

COWAT-Controlled Oral Word Association Test, TMT-Trail Making Test (Part B), FAI-Free Androgen Index, Free T-Free Testosterone, Total T-Total Testosterone, *=<0.05, **=<0.01
APPENDIX T

Scatterplot Showing Associations between Body Mass Index and Hospital Anxiety and Depression Scale- Depression Subscale Score In the Polycystic Ovarian Syndrome (N = 53) and Control (N = 50) Groups at Baseline.
Scatterplot Showing Associations between Body Mass Index and Hospital Anxiety and Depression Scale- Depression Subscale Score in the Polycystic Ovarian Syndrome ($n = 53$) and Control ($n = 50$) Groups at Baseline.
APPENDIX U

Correlations between Ferriman-Gallwey (FG) Score and Testosterone Levels, Mood and Anxiety in Polycystic Ovarian Syndrome (n = 27)
Correlations between Ferriman-Gallwey Score and Testosterone Levels, Mood and Anxiety in Participants with Polycystic Ovarian Syndrome (n = 27)

<table>
<thead>
<tr>
<th></th>
<th>Ferriman-Gallwey (Hirsutism) Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAI levels (pg/ml)</td>
<td>0.021</td>
</tr>
<tr>
<td>(n = 24)</td>
<td></td>
</tr>
<tr>
<td>Free Testosterone</td>
<td>0.021</td>
</tr>
<tr>
<td>levels (pg/ml)</td>
<td></td>
</tr>
<tr>
<td>(n = 23)</td>
<td></td>
</tr>
<tr>
<td>Total Testosterone</td>
<td>0.280</td>
</tr>
<tr>
<td>levels (ng/ml)</td>
<td></td>
</tr>
<tr>
<td>(n = 26)</td>
<td></td>
</tr>
<tr>
<td>HADS-D score</td>
<td>0.128</td>
</tr>
<tr>
<td>at baseline</td>
<td></td>
</tr>
<tr>
<td>(n = 27)</td>
<td></td>
</tr>
</tbody>
</table>

*Spearman’s r-value included in the table above, FAI= Free Androgen Index, HADS-D= Hospital Anxiety and Depression Scale-Depression sub score.*
APPENDIX V

Boxplot showing an Overlap between Free Androgen Index Levels across Polycystic Ovarian Syndrome ($n = 50$) and Control ($n = 53$) Groups
Boxplot showing an Overlap between Free Androgen Index Levels across Polycystic Ovarian Syndrome ($n = 50$) and Control ($n = 53$) Groups